UNIVERSITY OF LIVERPOOL

Clinical and Laboratory Features of HIV/AIDS in the Kingdom of Saudi Arabia

Thesis submitted in accordance with the requirement of the University of Liverpool for the degree of Doctor in Philosophy

Wail Bajhmoum

June 2015

Dedication

To:

Maria, Alaa, Leen and Lama.

My life, love and future.

Wail

Abstract

Introduction

There are insufficient data on the epidemiology and clinical features of HIV in the Middle East. WHO statistics show the Kingdom of Saudi Arabia (KSA) to be one of the least affected countries globally. However, the Saudi National Program for HIV Control reported a 34.6% increase in cases in 2008 from the previous year. Jeddah region has the highest proportion of HIV cases in KSA (40%). Infection risk data are not always complete and coinfection rates have not been studied. The first part of these studies included a retrospective longitudinal case review of all patients attending the Jeddah clinic to obtain a clearer view of clinical and epidemiological features of that population

There are few publications about resistance to antiretroviral therapy (ART) in the Arabian Peninsula, including KSA, and most are heavily biased towards assessment of patients with sequential treatment failure. Wider access to resistance testing has only become available recently and baseline local resistance patterns are largely unknown. The aim of the second part of the study was to determine patterns of ART resistance in a systematic fashion in treatment-naïve HIV positive Saudi patients and to document the presence and frequency of novel resistance HIV markers in Jeddah.

Study Part 1. Clinical features and epidemiology of HIV and coinfection with TB and/or viral hepatitis in a large clinic in Jeddah, Kingdom of Saudi Arabia

Aims: To describe demographic and clinical features of HIV infection in clinics and hospitals in Jeddah and to document prevalence and risks for coinfections with tuberculosis (TB) and/or hepatitis

Methods: Retrospective study including all HIV positive Saudi adults attending the main treatment centre in Jeddah in the 11 years (2000-2010). Data were systematically collected from case files and summarised. Statistical comparisons included univariate analyses with a p value <5% considered significant.

Results: 1383 HIV positive adults were reviewed, median (range) age 40 (18-86) years, of whom 1026 (74.2%) were male. Risk factors included heterosexual transmission in 709 (51.3%), men having sex with men (MSM) in 264 (26%), blood products in 148 (10.7%), injecting drug use (IDU) in 97 (7%) and not identified in 165 (11%). The predominant clinical presentation was with respiratory symptoms 611 (44%), followed by gastrointestinal manifestations in 312 (22%), while 29% (408) were asymptomatic. Past or present TB coinfection (clinical and/or radiology) was found in 208 (15%); 59 (5.3%) had hepatitis B serology positive (HBsAg positive) and 82 (7.4%) had hepatitis C coinfection (antibody positive). TB was associated with IDU (RR 1.67 (CI 1.13-2.41) p< 0.01) and having been in prison (RR 1.83 (1.18-2.85) p< 0.01) and these two risk factors were closely linked themselves. HBV coinfection was not linked with IDU (RR 1.89 (0.93-3.85) p=0.08) but was linked to being in prison (2.38 (1.25-4.54) p <0.01), while HCV was

strongly linked with IDU (RR 4.22 (2.71-6.57) p<0.01) and MSM but not with imprisonment (RR 1.94 (1.04-3.63) p=0.07)

Conclusion:

HIV/AIDS and related coinfections are medical problems in Saudi Arabia with many social challenges. The Saudi National Program for HIV Control actively addresses prevention of HIV and provision of high quality care for those affected. More detailed studies are needed on clinical patterns in outpatient and inpatient settings and on locally appropriate prevention programmes in high risk groups.

Study Part 2. HIV resistance in ART naïve patients in a large treatment centre in Jeddah, Kingdom of Saudi Arabia

Aims: To document the prevalence and types of ART resistance in ART naïve HIV patients in Jeddah, and to compare the efficacy of Next Generation Sequencing (NGS) with standard (Sanger) genotypic methods.

Methods: Plasma samples were collected from all ART-naïve patients sequentially attending the HIV clinic at King Saud Hospital, the main HIV treatment centre in Jeddah, between November 2012 and February 2013. Plasma was saved and HIV protease (Prot) and reverse transcriptase (RT) regions were sequenced according to routine in-house diagnostic protocols (Sanger) at Liverpool Specialist Virology Centre. The Stanford database was used for interpretation of resistance profiles. Neighbour-joining phylogenetic analysis was performed on samples with adequate sequence data for both Prot and RT. NGS sequencing was later performed at the Public Health England reference laboratory in Colindale.

Results: Blood samples were collected from 109/116 (94%) eligible patients approached to join the study. 71 (65%) were male and 52 (48%) had been diagnosed with HIV within the last 6 months. HIV RNA was successfully amplified from 96, and sequence data obtained from 93 Prot amplicons and 87 RT amplicons by Sanger and 105 by NGS. Mutations at putative resistance sites for NNRTI NRTI and PI were detected by Sanger in 9/87 (10.3%), 1/87 (1.1%) and 6/93 (6.5%) and 24/105 (22.9%), 6/105 (5.7%) and 34/105 (32.4%) respectively by NGS. Those with significant potential to confer resistance to NNRTI were found in 9/87 (10.3%), with resistance to NRTI in 1/87 (1.1%) and PI in 6/93 (6.5%). 4.5% of the samples showed resistance to efavirenz and nevirapine. The most common HIV-1 subtype was C (38%); although a cluster of CRF2_A (7%) was also prominent.

Conclusion: Clinically significant resistance is emerging (16 %) in this population. A variety of other markers included some clustering suggesting local transmission of primary resistance. The results are probably generalizable in KSA and we recommend the introduction of routine resistance testing for all HIV positive patients in the region before starting ART.

Overall:
These findings provide evidence for introduction of several changes to enhance the National HIV/AIDS programme in the Kingdom of Saudi Arabia.

Acknowledgements

This work could not be completed without the assistance and support of many people. I would like to express my deep thanks and appreciation to Dr. Nick Beeching for his endless support, guidance and close supervision. Thank you for the great organizing of every detail of this study along the past years. This work could not be done without your meticulous supervision.

Also I am greatly thankful to Dr. Anu Chawla my second supervisor for her advice and contiguous care.

Dr. Mark Hopkins, thank you very much for your cooperation and support in lab work. Professor Anna Maria Geretti, Professor David Lalloo and Professor Brian Faragher thank you all very much for your care and advice. Also I'm grateful to Colindale Centre for their assistance in processing samples for next generation sequencing (NGS) particularly Dr. Jean Lutamyo Mbisa, Acting Head, Antiviral Unit Virus Reference Department.

To all members of the staff of King Saud Hospital in Jeddah, I can't thank you enough. I'm also greatly thankful to Saudi Ministry of Health in Riyadh for the care and useful support particularly Dr. Ziad Memish, former Deputy Minister for Saudi Public Health.

Finally I'd like to express my deep appreciation to my family and friends, specially my lovely mom Maria and wonderful wife Alaa.

Contents **Page**

Contents **Page**

Contents **Page**

Content **Page**

Contents **Page**

Contents **Page**

Contents **Page**

Acronyms/Abbreviations

AIDS	Acquired Immunodeficiency Syndrome
ART	Antiretroviral Therapy
ARV	Antiretroviral drugs
BBV	Blood borne virus
CDC	Centers for Disease Control and Prevention
CD4	Cluster of Differentiation 4 lymphocyte
CI	Confidence Interval
CMV	Cytomegalovirus
CNS	Central Nervous System
DNA	Deoxyribonucleic Acid
EBV	Epstein-Barr Virus
HAV	Hepatitis A Virus
HAART	Highly Active Antiretroviral Therapy
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
IATA	International Air Transport Association
IDU	Injecting Drug User

KSA	Kingdom of Saudi Arabia
KSH	King Saud Hospital
MENA	Middle East and North Africa
MOH	Ministry of Health
MSM	Men who have Sex with Men
MTA	Material Transfer Agreement
NAP	National Saudi AIDS Programme
NGS	Next Generation Sequencing
NNRTI	Non-Nucleoside/Nucleotide Reverse Transcriptase Inhibitor
NRTI	Nucleoside/Nucleotide Reverse Transcriptase Inhibitor
OI	Opportunistic Infection
OR	Odds ratio
PCR	Polymerase Chain Reaction
PI	Protease Inhibitor
PLWHIV	People living with HIV
RAM	Resistance Associated Mutation
RNA	Ribonucleic acid
RR	Relative Risk
SD	Standard Deviation
SDRD	Stanford HIV Drug Resistance Database
SPSS	Statistical Package for the Social Sciences
STI	Sexually Transmitted Infection
TAMS	Thymidine Analogues Mutations
TB	Tuberculosis
UNAIDS	Joint United Nations Programme on HIV/AIDS

UNGASS	UN General Assembly Special Session
VDRL	Venereal Disease Research Laboratory
WHO	World Health Organisation

Tables

Figures

Chapter 1

Introduction

1. 1. Introduction:

1.1.1 Global view

The HIV/AIDS pandemic is one of the most important medical problems globally over the past 30 years. The Human Immunodeficiency Viruses (HIV) are retroviruses of simian origin that transcribe ribonucleic acid (RNA) into cell deoxyribonucleic acid (DNA) and integrate the genome in the human host. There are two main HIV types which cause human disease: HIV-1 and HIV-2.

Phylogenetic sequence analysis from apes and humans suggest that the infection and transmission of HIV to humans started as early as 1930. 50 years later the first case of HIV/AIDS was described in 1981 in the US (Centers for Disease Control (U.S.) 1981). HIV/AIDS had been recognised in all world regions by 1985 (Peters et al. 2013).

Infection is spread by sexual contact, transfusion of blood products or organs, by needles in healthcare practice or by injecting drug use (IDU) and from mother to child. Once HIV enters the host CD4+ T-

lymphocyte, it converts its RNA to DNA by using reverse transcriptase enzyme. The cell is then used to produce more HIV viruses and the cycle continues. As infection progresses, the number and function of the CD4 cells decreases and the amount of HIV in the body increases. The person becomes increasingly susceptible to infections, some of which are otherwise rare, and to a variety of tumours and metabolic problems. This late stage of HIV infection is often known by its earlier title AIDS, or Acquired Immunodeficiency Syndrome (AIDS). Without specific treatment, AIDS is fatal although the time to progression varies in different people.

The rates of new HIV infections affect some world regions more than others, predominantly in central Asia, Sub-Saharan Africa and Eastern Europe. Globally, Africa continues to be the most affected region, with increasing rates in Asia. HIV/AIDS has a strong impact on individual patient's social life, economy and development and a huge social and economic impact on countries in which it has increased. Efforts against HIV/AIDS should continue and should be focused to control this global threat (Piot et al. 2001).

The introduction of antiretroviral therapy (ART) has dramatically improved the clinical management of HIV/AIDS patients. Studies performed soon after introduction of Highly Active Antiretroviral Therapy (HAART) with three classes of antiretroviral drugs (ARV) in

1996 showed a remarkable decline in mortality and morbidity of up to 85% in treated patients (Palella et al. 1998; Flepp et al. 2001).

In 2013, the World Health Organization (WHO) and the Joint United Nations Programme on HIV/AIDS (UNAIDS) estimated that globally there were 35.3 million people living with HIV (PLWHIV). In the same year, there were 2.3 million newly diagnosed HIV/AIDS cases (UNAIDS 2013).

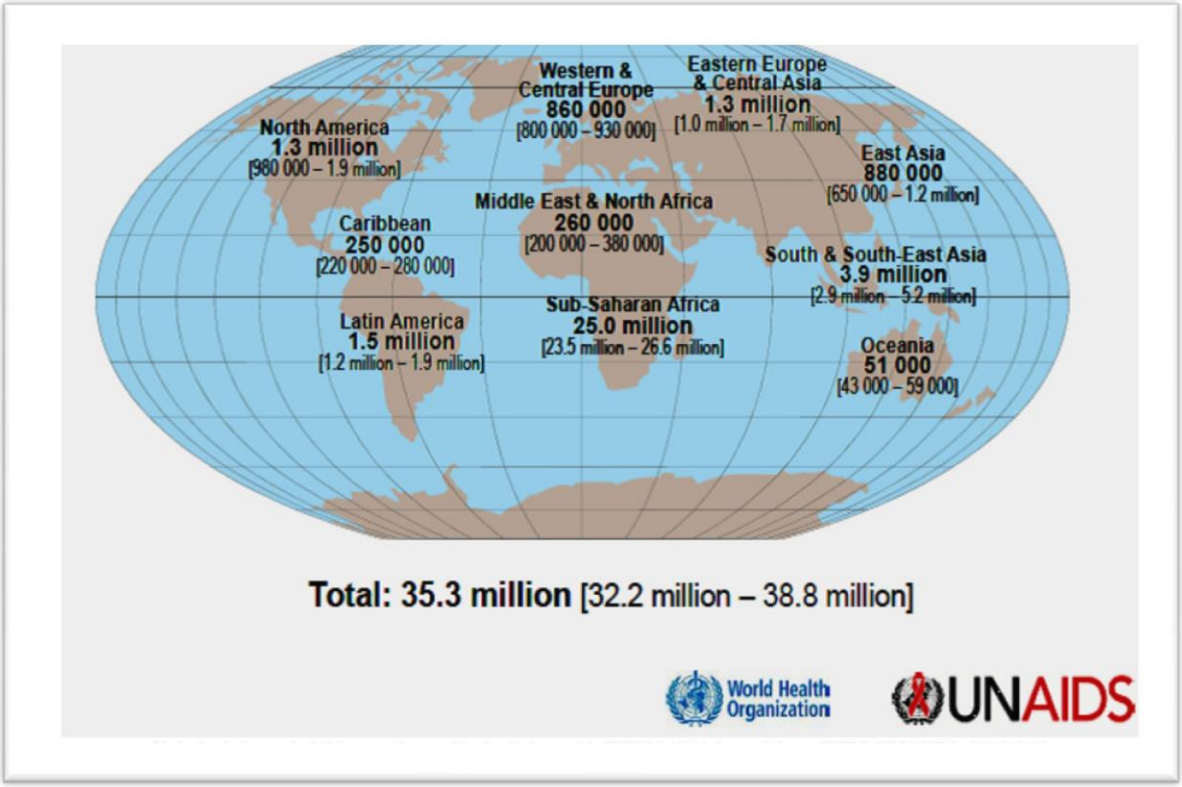


Figure (1-1) Global HIV/AIDS numbers in 2011 (UNAIDS 2013)

1.1.2 Regional view

In the Middle East and North Africa (MENA) data on the epidemiology of HIV/AIDS and behavioural and social information are incomplete and need to be improved for better understanding of the situation and to inform quality prevention and treatment programmes. Although several countries have taken forward steps to improve HIV information systems, passive reporting remains the primary mechanism for obtaining evidence on epidemiological and behavioural trends in the region (Shawky, Soliman & Sawires 2009). This study also reflected the effect of social constraints on the HIV/AIDS situation in the MENA region. In conservative societies found in Saudi cities, men are more independent than women and thus, men are more able to experience sexual relations than women. This may explain the predominant male gender bias in HIV in the Region. Women face many barriers to accessing accurate information about HIV, because in this society they are not allowed to be sexually experienced. Societal ideals for relations between women and men may increase the risk for of infection for both genders. However, in the last 5 years the female to male ratio has increased, possibly because of improved cultural mobility and as a result of effective reachout of local HIV programmes. In addition, improvements in the systems of notification and surveillance in the Region have started to reflect the true local prevalence of HIV infections.

There is particularly little information about HIV in the Middle East. Until recently, most countries in the Region suppressed information about HIV. In the past this was particularly because HIV was presented to the population as a disease caused by bad sexual morals. In addition, HIV testing has been considered a very sensitive issue especially in monitoring high risk groups such injecting drug use (IDU) and men who have sex with men (MSM), which limits screening strategies in this region (Obermeyer 2006). His review of HIV/AIDS was performed in 2006 and concentrated on the whole MENA region, ignoring the important detail that societies in the Middle East are culturally very different from those in North Africa. Furthermore, there are many cultural differences within the Middle East Region itself. With development of life-saving therapy over the past decade or more, some countries in the Region have acknowledged the importance of examining various aspects of HIV in their own setting. This includes identifying local risk behaviour and high risk groups, the social behaviour of these groups and the responses of doctors and other health care workers.

Moussavi et al studied knowledge about HIV/AIDS in Iranian adolescents in 2007 and showed that they form a particularly important target group for primary prevention of HIV in the community. The study was carried out by anonymous questionnaires administered to 1227 Iranian students. The students reported that

television (84%) and school teachers (66%) were the best sources of HIV/AIDS information, while parents (27%) and school books (15%) were least informative. Most students knew that heterosexual intercourse (90%) and shared intravenous needles (94%) can cause HIV infection; however, many misconceptions were revealed. Only 53% were aware that condoms protect against infection through sexual intercourse. More effective school-based HIV/AIDS education is needed in Iran. This study identified misconceptions about viral transmission and the availability of a cure that highlights the need for further HIV/AIDS prevention among adolescents. Descriptive results contribute to a larger process by which barriers of silence are broken and foundations provided for targeted interventions. Future studies in Iran must take into consideration the ways in which cultural and religious value systems impact on lifestyle and access to information. Schools are particularly useful settings for HIV/AIDS interventions because they provide an existing community based infrastructure, while reaching a sizeable target population during a critical period. (Moussavi et al 2007)

In 2013 a similar study in Turkey assessed the knowledge about HIV/AIDS in two high schools in Kırıkkale, Turkey (Aylikçi et al. 2013). Most of the 473 students (92.2%) had some knowledge about HIV/AIDS prior to the study. 27% believed that there is a cure for AIDS and 64% believed that HIV can be avoided by using condoms during sexual contact (Aylikçi et al. 2013).

In 2014, Al-Malki published an interesting study looking at the knowledge of STI among students at Taif University, Saudi Arabia. All 400 participants were adults aged 18-30 year old. 27.7% obtained their knowledge about sexually transmitted infections (STI) from the internet. 36.8% knew that an infected mother could transmit a sexual disease to her infant during labour. The vast majority of students (98.5%) were aware of HIV/AIDS as a STI. This study also showed associations of better understanding of STI with being married and having a good parental educational level. The study concluded that education and religious programmes should be given early on in secondary schools. In addition, premarital screening is important for controlling STI in the kingdom (AL-Malki 2014). This study reflected the knowledge of a specific group and cannot be generalised over the different populations in the Kingdom. Even though this study examined some aspects of knowledge among these students, more critical questions could have been asked especially about high risk sexual behaviours in such a restricted and conservative society.

In the Kingdom of Saudi Arabia (KSA), there has been a major change in official attitudes. The Ministry of Health (MOH) has established a comprehensive programme for monitoring the epidemiology of HIV/AIDS and for publishing the data in an open format. Treatment centres and management protocols have been established in every part of the Kingdom. Public information and prevention programmes have continuously been introduced.

However, there has been little assessment of the clinical features or the effects of coinfection with other pathogens such as hepatitis B (HBV) or C (HCV) or tuberculosis (TB) and there are no published data on clinical audit. Very few data are available in the whole Region or the Kingdom about resistance to antiretroviral regimens, which is essential to guide treatment policies.

The treatment centre with the largest population of HIV positive patients is in Jeddah (Kabbash et al. 2012). In addition, risk factors such as injecting drug use (IDU) are known to be present in Jeddah, although several studies have previously shown a relatively low prevalence of HIV in these patients (Kabbash et al. 2012).

1.1.3 Aims:

The main aims of this work were to:

1. Review the epidemiology of HIV in the Middle East and in particular in the Kingdom of Saudi Arabia (KSA).
2. Review Regional and KSA data on coinfection of HIV with hepatitis B, C and tuberculosis.
3. Describe the clinical presentation, demographic features and rates of coinfection in a large outpatient HIV clinic in Jeddah.
4. Investigate the prevalence and types of primary resistance to antiretroviral therapy in HIV positive patients in Jeddah.

1.2. Literature Review

This chapter provide a brief review of the global epidemiology of HIV and then detailed review of HIV in Arabian Peninsula. The review includes recent data on HIV epidemiology in the Region and in the Kingdom of Saudi Arabia. World regions were defined according to the WHO definition (UNAIDS 2013).

WHO HIV/AIDS surveillance

A wide range of surveillance methods and indicators have been used to monitor the HIV/AIDS epidemic by WHO in developing countries.

These include measures of disease occurrence (HIV incidence and prevalence indicators) as well as indicators of risk and impact.

- *Passive surveillance*: This is the routine reporting of cases of diseases reaching health care facilities for treatment or service. No special effort is made to find unsuspected disease incidence.

- *Active surveillance*: A search is conducted to find cases in the community, mainly through door-to-door surveys or by targeting specific high risk populations. This method also

includes the gathering of information from institutions and healthcare providers.

- *Sentinel surveillance*: This is a reporting system based on selected institutions or people who provide regular, complete reports on one or more diseases, ideally occurring in a defined attachment format.

The WHO has promoted sentinel surveillance as the method of choice for monitoring the spread of HIV in developing countries (Riedner & Dehne 1999). In addition, the occurrence of other infections has occasionally been used as a surrogate indicator of HIV spread in these countries. Therefore, surveillance of sexually transmitted infections (STI) and HIV associated diseases such as TB and herpes zoster (HZV) have been used as HIV infection markers in some populations (Riedner & Dehne 1999).

Surveillance in developing countries includes HIV and AIDS case reporting, sentinel sero-surveillance, and STI and behavioural surveillance (Riedner & Dehne 1999)

1.2.1 Global epidemiology.

The global prevalence of HIV-1 is 0.8%, this includes 34 million people living with HIV/AIDS and 1.7 million AIDS deaths in 2011 (UNAIDS 2011).

	Estimate	Range
People living with HIV/AIDS	34 million	31.4 – 35.9 million
Newly infected patients	2.5 million	2.2 -2.8 million
HIV/AIDS deaths	1.7 million	1.5 -1.9 million

Table (1-1) Global HIV estimates for 2011 (UNAIDS 2013)

WHO estimates for the epidemiology of HIV/AIDS according to the Region are summarised in the table (1-2).

Region	PLWHIV	Newly infected	Prevalence	AIDS deaths
Sub-Saharan Africa	23.5 million	1.8 million	4.9%	1.2million
North Africa and Middle East	300,000	37,000	0.2%	23,000
South and South East Asia	4 million	280,000	0.3%	250,000
East Asia	830,000	89,000	0.1%	59,000
Oceania	53,000	2,900	0.3%	1,300
Latin America	1.4 million	83,000	0.4%	54,000
Caribbean	230,000	13,000	1.0%	10,000
East Europe and Central Africa	1.4 million	140,000	0.2%	92,000
North America	1.4 million	51,000	0.6%	21,000
Western and Central Europe	900,000	30,000	0.2%	7,000
Global Total	34 million	2.5 million	0.8%	1.7 million

Table (1-2) Global HIV/AIDS epidemiology in 2011 (UNAIDS 2013)

The heterosexual route remains the most common mode of transmission in addition to male to male sex (MSM) and injecting drug use (IDU). The rates of new HIV infections are generally declining in most of the world's Regions but are increasing in certain Regions such as in Eastern Europe and central Asia.

1.2.1.1 ASIA:

Asia is the largest continent, with almost 60% of the global population. It has the second highest number of people living with HIV/AIDS after Africa. WHO UNAIDS estimated that 370,000 newly diagnosed HIV patients were living in East Asia in 2011 compared with 310,000 in 2001 (UNAIDS 2013) but this number has dropped to 280,000 newly diagnosed patients in 2013 (UNAIDS 2013)

1.2.1.2 Sub-Saharan Africa

The largest burden of HIV has been in Sub-Saharan Africa, accounting for around three quarter of the global death toll so far. The epidemic emerged in 1980's and in contrast to certain settings was largely spread by heterosexual sex and from mother to child at birth or in infancy. It was accompanied by a huge rise in tuberculosis cases in the 1990's. Mortality was high due to poor health infrastructure and lack of treatment in many countries. By 2001 there were 21 million PLWHIV which increased to 25 million in 2012 (UNAIDS 2013). However, there has been a drop in the number of newly diagnosed patients from 2.4 million in 2001 to 1.2 in 2012 (UNAIDS 2013).
The peak age group of diagnosis is younger in women than in men, and the prevalence of HIV in all young adults was estimated at 4.9% across the region in 2011. The incidence and epidemiology differ generally from North African setting, although there is an intermediate situation in the Sudan, close to KSA

1.2.1.3 East Europe and Central Asia

Eastern Europe and Central Asia is the only region where HIV prevalence clearly remains on the rise. UNAIDS estimated 1.4 million PLWHIV in this region (UNAIDS 2013). 110,000 newly diagnosed cases in 2008 (UNAIDS 2008) had increased to 140,000 in 2012 (UNAIDS 2013).

1.2.1.4 Middle East and North Africa (MENA)

In general the prevalence of HIV/AIDS prevalence in the Middle East and North Africa (MENA) Region is low. However, even in areas of low prevalence, at-risk populations in those countries may be heavily affected by the HIV epidemic (Abu-Raddad & Longini 2008).

Up to the present there has been little dissemination of information about HIV in the Middle East, as many countries in the Region used to suppress related data about HIV within their countries.

The most recent estimate of the number of people living with HIV/AIDS in the Middle East and North Africa region is about 260,000 in 2012 (UNAIDS 2013); the accuracy of this estimate is low because of poor and incomplete data. Publications from this region were limited especially from the Middle East countries and HIV/AIDS data were collected by notifying the cases as a part from the official HIV reports to WHO.

Country	Year	Prevalence	PLWHIV	AIDS deaths	Reference
Middle East & North Africa	2013	0.1%	260,000	17,000	(UNAIDS 2013)
Bahrain	2010	0.2% (2008)	NA	NA	(UNGASS Country Progress Report 2010)
Emirates	2012	0.2% (2008)	NA	NA	(UNAIDS 2008) and (UNGASS Country Progress Report 2012)
Iran	2013	0.2%	71,000	4,600	(UNAIDS 2013) and (UNGASS Country Progress Report 2012)
Iraq	2008	0.1%	NA	NA	(UNGASS Country Progress Report 2008)
Israel	2011	0.2%	3875	832	(WHO, key facts on HIV epidemiology in Israel and progress 2011)
Jordan	2011	0.2%	8,500	25	(WHO, KEY FACTS ON HIV EPIDEMIC IN ISRAEL AND PROGRESS 2011)
Kuwait	2010	0.1%	600	500	(UNGASS Country Progress Report 2010)
Lebanon	2013	0.1%	NA	NA	(UNAIDS 2013)
Oman	2012	0.1%	3,000	200	(UNGASS 2012)
	2013	0.1%	1,371	200	(UNAIDS 2013) and (UNGASS Country Progress Report 2012)
Qatar	2013	0.1%	NA	NA	(UNAIDS 2013)
Saudi Arabia	2014	0.1%	20,539	NA	(UNGASS Country Progress Report 2014)
Syria	2008	0.1%	500	200	(UNGASS Country Progress Report 2008)
Yemen	2013	0.1%	19,000	NA	(UNAIDS 2013)
Other Islamic Countries					
Egypt	2013	0.1	9,200	500	(UNAIDS 2013)
Sudan	2010	1.4	77,000 (2013)	25,000	(UNGASS Country Progress Report 2010) and (UNAIDS 2013)
Turkey	2010	0.1	N.A	N.A	(UNGASS Country Progress Report 2010)

Table (1-3) HIV data in the Middle East and selected neighbouring countries

(various sources)

PLWHIV: People live with HIV

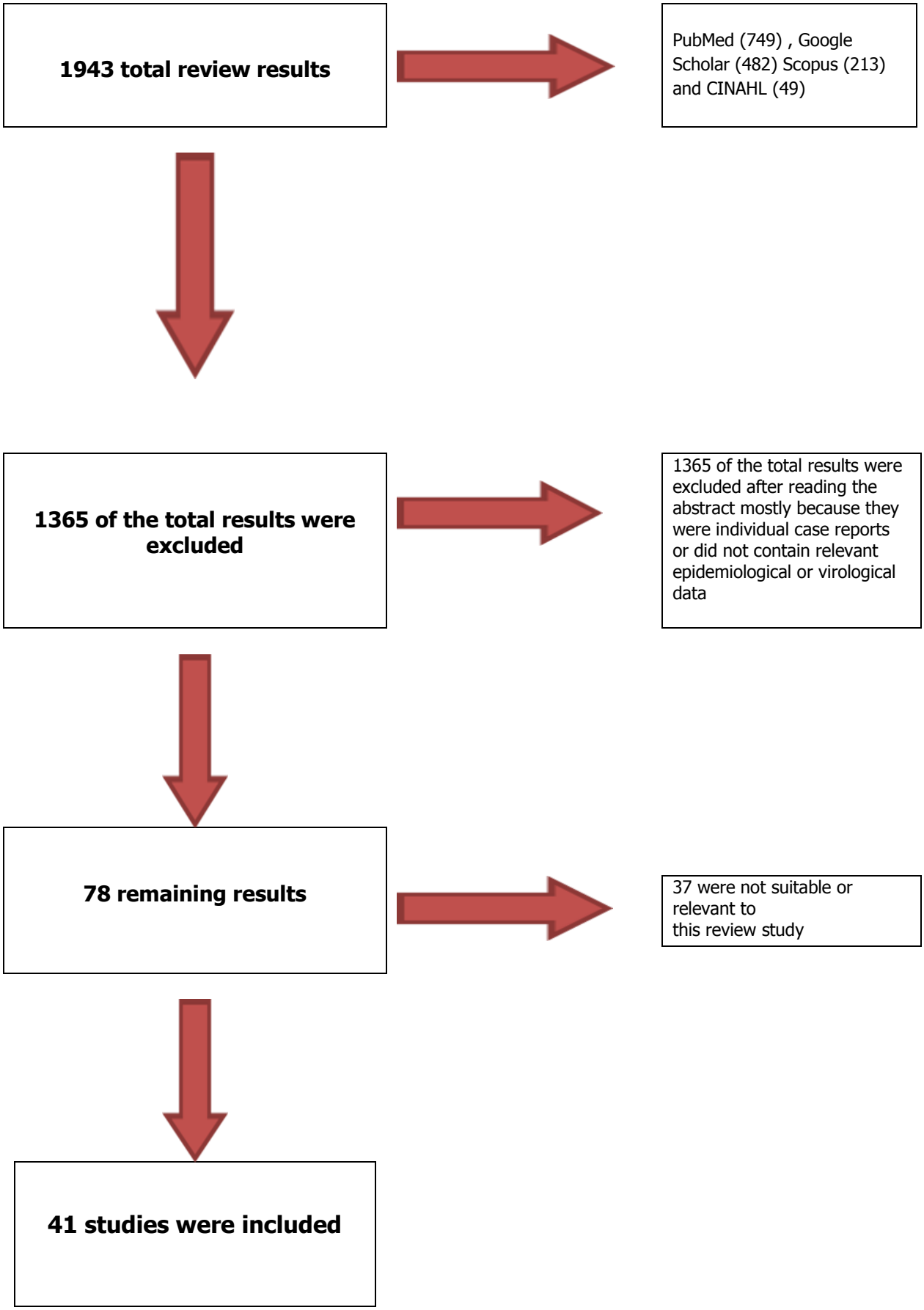
The HIV-related literature in the Region is patchy, emphasising the paucity of systematic surveillance efforts and the difficulty of enhancing activities that reduce risks, together with the challenges to measuring the HIV/AIDS epidemic in societies in this Region. There is also a need to ensure that diagnostic and therapy strategies are following best international practice.

In order to obtain a clearer picture of the epidemiology and clinical presentation of HIV and antiretroviral resistance in the Region a systematic literature review was performed. Large databases (PubMed, Scopus, Google Scholar and CINAHL) were investigated using a wide search strategy for the period 1981 to 2014. Combinations of keywords included HIV, AIDS, drug misuse, ART, ARV, coinfections, TB, hepatitis, HIV treatment naïve, resistance together with geographical terms such as Middle East, Saudi Arabia and each country in the Middle East and North Africa (MENA) WHO Region. The WHO website and UNAIDS websites were also consulted for any references to HIV and to ART resistance. Abstracts were initially reviewed, the relevant papers and reviews were obtained and the reference lists were consulted to identify further sources. The content indexes of regional scientific journals were also reviewed including the *Saudi Medical Journal*, *Annals of Saudi Medicine* and *Journal of Infection and Public Health*. Furthermore, the websites of the Ministry of Health (or equivalent) for each country were reviewed

to obtain reports related to HIV epidemiology or treatment. These reports were then reviewed.

The review started at the beginning of work and was repeated in March 2015 to include more recent publications.

1493 results were found in the following databases: PubMed (749), Google Scholar (482), Scopus (213) and CINAHL (49). 1365 of the total results were excluded after reading the abstract mostly because they were individual case reports or did not contain relevant epidemiological or virological data. Of the remaining 78, 37 were not suitable or relevant to this review study. Suitable results from 41 studies were included (Table 1-4).


1943 total review results
PubMed (749) , Google Scholar (482) Scopus (213) and CINAHL (49)
1365 of the total results were excluded
1365 of the total results were excluded after reading the abstract mostly because they were individual case reports or did not contain relevant epidemiological or virological data
78 remaining results
37 were not suitable or relevant to this review study
41 studies were included

Country or region	Year of publication	Authors
Bahrain	1994	Al-Haddad et al
Bahrain	1997	Al-Haddad et al.
Bahrain	2002	Ehrahim et al
Bahrain	2014	Saeed et al.
Iran	2009	Fallahzadeh et al.
Iran	2012	Rahimi-Movaghar et al.
Israeli Arab	2005	Chemtob et al.
Israel (Palestine)	2004	Chemtob et al.
Israel (Palestine)	2013	Mor
Israel (Palestine)	2014	Zohar et al.
Jordan	2010	Bakri et al.
Kuwait	2000	Al-Owaish et al.
Kuwait	2012	Akhtar et al.
Lebanon and Suadi Arabia	2005	Al-Mazrou et al.
Lebanon and Suadi Arabia	2006	Traboulsi et al.
Lebanon	2010	Naba et al.
MENA	2004	Sufian
MENA	2006	Obermeyer
MENA	2009	Shawky et al.
MENA	2011	Mumtaz et al.
MENA	2014	Bozicevic et al.
Morocco	2008	Ministry of Health HIV Report
Oman	1999	Scrimgeour et al.
Oman	2012	Balkhair et al.
Oman	2004	Al Dhahry et al.
Saudi Arabia	1993	Ellis et al.
Saudi Arabia	2004	Alrajhi
Saudi Arabia	2004	Madani
Saudi Arabia	2004	El-Hazmi
Saudi Arabia	2005	Al-Mazrou et al.
Saudi Arabia	2010	Al-Jabri et al.
Saudi Arabia	2010	Alothman et al.
Saudi Arabia	2010	Jamjoom et al.
Saudi Arabia	2012	Kabbash et al.
Syria	2013	Seal
Turkey	2009	Alim et al.
Turkey	2013	Aylikçi et al.
Turkey	2014	Agacfidan
UAE, Dubai	2011	Al-Dabal et al.
Yemen	2007	Lambert
Yemen	2014	Mirzazadeh et al.

Table 1-4 Publications used in this review of epidemiology of HIV in MENA region

In 2011, 14 countries from the MENA region reported 4263 new HIV cases of which 66.8% were men. Heterosexual sex was the most common reported route of transmission. This could be due to under reporting of male-to-male transmission and more frequent testing of men than women (Bozicevic, Riedner & Haghdoost 2014). This paper reviewed the country reports to WHO about HIV/AIDS overview from the countries in the Eastern Mediterranean Region (WHO EMR). Most of these countries depend on passive surveillance for HIV data reporting to WHO. However, the Saudi National AIDS Programme (NAP) enhanced active surveillance, targeting high risk groups and certain populations in the community (NAP 2014).

HIV/AIDS transmission in the region is also recognised in certain high risk groups such as injecting drug users (IDU) and in men having sex with men (MSM), but heterosexual transmission is still the most common route of infection reported in the MENA WHO region (Obermeyer 2006).

Country	Year	IDU	MSM	Prisoners	Reference
Bahrain	2011	0.3%	NA	NA	(UNAIDS 2011)
Egypt	2013	6.7%	4.1%	NA	(UNAIDS 2013)
Emirates	2013	NA	NA	NA	(UNAIDS 2013)
Iran	2013	13.6%	15.9%	2.0%	(UNAIDS 2013) and (UNGASS Country Progress Report 2012)
Iraq	2013	NA	NA	NA	(UNAIDS 2013)
Israel	2011	10.2%	36.7%	NA	(WHO, KEY FACTS ON HIV EPIDEMIC IN ISRAEL AND PROGRESS 2011)
Jordan	2010	2.0%	NA	NA	(UNGASS Country Progress Report 2010)
Kuwait	2013	NA	NA	NA	(UNAIDS 2013)
Lebanon	2012	5.7%	20.0%	NA	(UNGASS 2012)
Oman	2013	1.1% (2013)	14.1%	NA	(UNAIDS 2013) and (UNGASS Country Progress Report 2012)
Qatar	2013	NA	NA	NA	(UNAIDS 2013)
Saudi Arabia	2014	NA	NA	1.6%	(UNGASS Country Progress Report 2014)
Syria	2013	0.5%	NA	NA	(UNAIDS 2013)
Turkey	2014	NA	8.9%	NA	(Agacfidan 2014)
Yemen	2013	NA	5.9%	NA	(UNAIDS 2013)

Table (1-5) Prevalence of high HIV in risk groups in some Middle East and North African countries (IDU, Injecting drug users, MSM, men who have sex with men)

There are usually two known epidemiological patterns of HIV/AIDS transmission in the Middle East and North Africa. First, patients are infected with HIV while they are travelling out of the country and they then transmit to their sexual partner. The second is transmission within high risk populations, which also leads to sexual partner infection. In the early years of HIV epidemic in the region,

transmission occurred in recipients of blood products including renal patients, haemophiliacs and others (Madani 2004).

Most countries in the MENA region are still slow to develop optimal HIV/AIDS strategies and treatment programmes. Although antiretroviral therapy (ART) is provided at low cost or even in some countries free of charge, only about 5% of those who needed the therapy in MENA region were actually receiving it by the late 2005 (Obermeyer 2006). Only 14% of those needing antiretroviral therapy had received treatment during 2008 and treatment coverage in the region was less than 50% of the international average for low and middle income countries (WHO, UNCF, UNAIDS, 2009). However, this does not apply in all countries of the MENA Region especially in resource rich countries like Saudi Arabia and the Arabian Gulf states. ART is provided to all diagnosed HIV patients free of charge in addition to providing full medical care. It is, therefore, important to differentiate between resource rich countries and resource poor countries in the Region.

But there have been some improvements in some parts of the Region. For example, HIV counselling and testing in Yemen had increased 18 times in 2006 compared to the previous year (UNGASS country progress report 2007). In the Morocco also counselling and testing for HIV had increased according to Morocco Health Ministry HIV report in 2008, increasing about 24 times from 1500 people in 2001 to 35458 people in 2007.

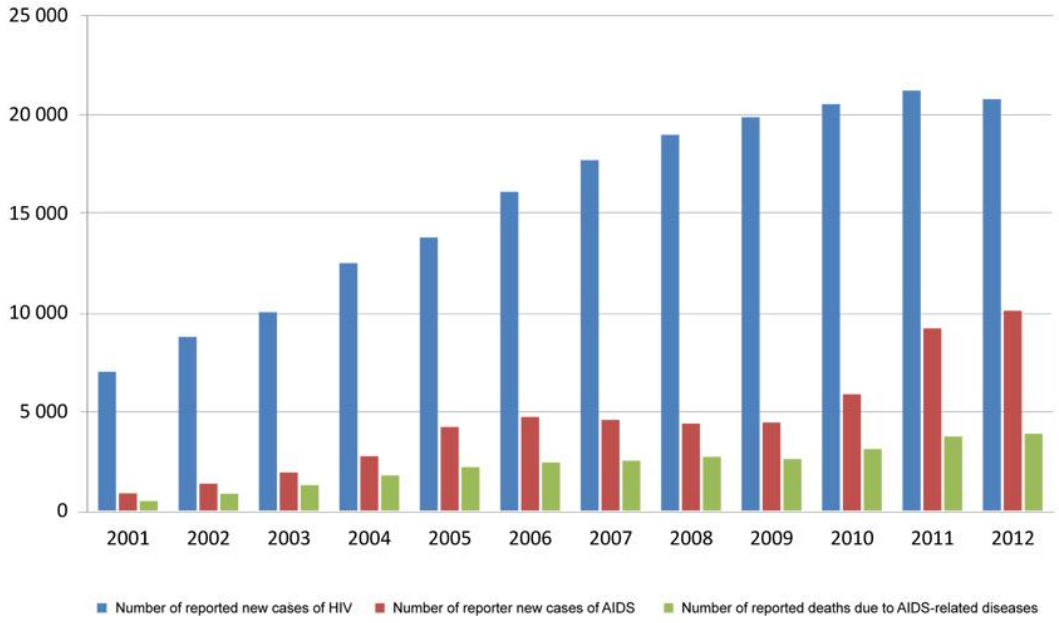


Figure (1-2) Reported HIV cases in MENA (new, AIDS cases and deaths) from 2001-2012 (UNAIDS 2013)

No country in the MENA Region has performed fully systematic surveys of HIV prevalence and incidence. In 2003 the World Bank reported that the assumed data of low rates of HIV/AIDS in the Middle East and North Africa led to underestimation of the real state of the problem. As a result, authorities in these countries gave a lower priority to HIV/AIDS. It was recommended that efforts should be made to improve HIV data and studies to reflect the actual situation, in order to achieve the best for the HIV population in this area (Sufian 2004)

In addition, data and literature on HIV/AIDS in Arab populations is very limited. Discussion of subjects related to HIV/AIDS is highly sensitive in this society and this is considered to be the main cause of the data shortage in this region. However, in the past 5 years, the quantity and quality publications of HIV/AIDS data in the region have improved. Moreover, many national plans and international cooperations have been initiated to improve medical strategies in fighting HIV.

1.2.2.1 Bahrain

Al-Haddad et al reviewed 241 male IDU patients followed at the drug rehabilitation clinic of the Psychiatric Hospital in Manama, Bahrain for a year (Al-Haddad et al. 1994). They looked at the risk factors for HIV infection among the IDU. Patients provided demographic and behavioural information based on a questionnaire. 21 % of IDU patient were HIV positive (Al-Haddad et al. 1994), much higher than the 0.3%reported in 2011 (UNAIDS 2011)

Three years later, Al-Haddad et al reviewed all HIV patients in Bahrain within the period from 1986 to 1996. There were 378 HIV diagnosed, of which 51% were foreign nationals. 38.8% of Bahraini HIV patients were IDU and the heterosexual route of transmission found in 45.7%. The male to female ratio for Bahraini nationals was 10:1 (Al-Haddad, Baig & Ebrahim 1997).

Pneumocystis jirovecii pneumonia was the commonest (50%) opportunistic infection (OI) , followed by tuberculosis (21%). This was one of the earliest OI reviews of HIV in Bahrain and in the whole Region. Only 378 patients were included and more than half were non-nationals (Al-Haddad, Baig & Ebrahim 1997).

Ehrahim et al from the Arabian Gulf University, Manama, Bahrain studied the microbial infections most commonly found in Bahraini HIV patients. They reviewed 67 HIV positive Bahraini patients seen between May 1997 and November 1998 retrospectively. 31.3% of patients had a CD4 count less than 100 cells/mm^3, 7.5% had CD4 100-200 cells/ mm^3, 37.3% had 201-500 cells/ mm^3 and 23.9% had counts greater than 500 cells/ mm^3. *Staphylococcus epidermidis* was found in 14.3%, *Pseudomonas aeruginosa* infection in 9.5% and *Haemophilus influenzae* infection found in 9.5%. *Pneumocystis jirovecii* was found in 9.5% and herpes simplex in 4.8 % (Ehrahim et al. 2002).

In 2014 Saeed et al reviewed opportunistic infections in HIV positive patients in Bahrain over 4 years. This retrospective study was based on laboratory records from a major hospital in Bahrain, from January 2009 to May 2013. *Staphylococcus aureus* was the commonest infections (9.8%) and TB infection was present in 3.6%. *Pneumocystis jirovecii* pneumonia (PCP) was observed in 5.1% (Saeed, Farid & Jamsheer 2014).

1.2.2.2 Iran

In another neighbouring Islamic country, Iran, considerable data have been published, including especially data on high risk groups such as prisoners and IDU. Fallahzadeh et al reviewed the HIV/AIDS data in Iran between 1986 and 2006, stating that "surveillance of HIV/AIDS in Iran has faced many challenges, such as under-reporting and difficulties in reaching high-risk groups, leading to an inaccurate and incomplete epidemiological profile" (Fallahzadeh, Morowatisharifabad & Ehrampoosh 2009).

Rahimi et al reviewed many studies in Iran and estimated the HIV/AIDS prevalence in IDU over the period of ten years starting from 1998. 22 studies were reviewed and this included a total of 3916 IDU. 10 studies were done in prisons and the other 12 studies were conducted in medical centres. The systematic review found that HIV/AIDS prevalence among IDU had increased after 2005 to 18.4% compared to 8.7% before 2005. They recommended improving HIV reduction strategies for such patients (Rahimi-Movaghar et al. 2012). However, this study, like many others in Iran, concentrated on one high risk group (IDU). Therefore the results cannot be generalised for

all HIV patients in Iran and more studies are needed to include all risk groups for HIV infection.

In 2013 the prevalence of HIV in Iran was 0.2% with 71,000 PLWHIV and 4,600 deaths due to HIV infection (UNGASS Country Progress Report 2012; UNAIDS 2013)

1.2.2.3 Jordan

There are few data on HIV in Jordan, UNAIDS has estimated that there were about 2000 people liveing with HIV/ (UNAIDS 2010). Figure 1-3 summarises the modes of HIV transmission in 194 patients in Jordan 1996-2008 (Bakri et al. 2010). This study showed the most common route of HIV transmission to be heterosexual, in keeping with other countries in the Region. Transmission by transfusion of blood or blood products was found in about 30% of the cases from 1996 to 2008. This proportion has fallen since then, as in the recent 5 years all blood donors in Jordan and other Middle East countries are tested for HIV before donation. The proportion of MSM in this study may be underestimated or hidden for various social and religious reasons. Further studies were recommended with a large and more representative sample size to reflect the actual prevalence and routes of transmission of HIV/AIDS in this country.

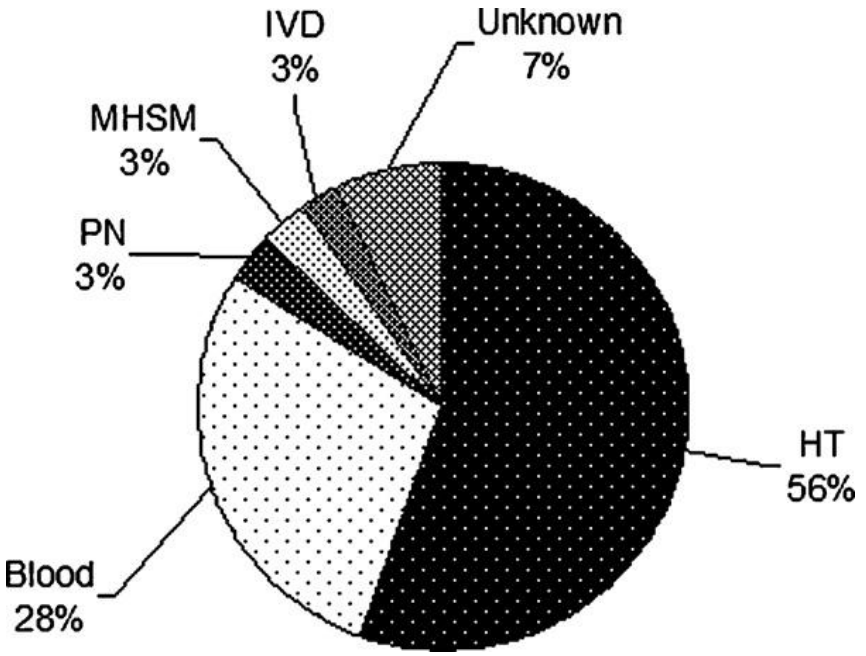


Figure (1-3) Modes of HIV transmission among 194 Jordanian patients (Bakri 2010)

HT, heterosexual; MHSM, men having sex with men; IVD, intravenous drugs; PN, perinatal

1.2.2.4 Kuwait

In 2000 Al-Owaish et al found that of 1984 HIV patients in Kuwait, 76% were in the age group 15-34 years. Their study indicated that reported HIV/AIDS cases in Kuwait had increased over the time. They also showed that 53% of those patients were married and that most were illiterate (Al-Owaish, Anwar et al. 2000).

In 2012, Akhtar and Mohammad found that the prevalence of HIV among migrant workers in Kuwait was 21 in 100 000, and that TB affected 198 patients in 100 000 (Akhtar & Mohammad 2012).

However, the study covered the previous 10 years and only included migrant workers coming to Kuwait and did not reflect HIV in Kuwaiti citizens. However, it is one of very few studies in the Region to report on migrants and expatriates as these are usually deported as soon as their HIV status is diagnosed. There was no description of the clinical conditions or hospital admissions in this study. There are no data on clinical features or the epidemiological situation of HIV in Kuwait. It has been estimated that Kuwait still has a low HIV prevalence of 0.1%. The cumulative reported HIV diagnoses from 1986 to 2011 was 206 cases (UNAIDS 2013).

1.2.2.5 Lebanon

HIV/AIDS cases were first diagnosed in Lebanon in the early 1980's, and this nearby country has similar features of HIV/AIDS to Saudi Arabia, with the most common route of transmission being heterosexual (Al-Mazrou et al. 2005). There are similarities in the high risk groups and in the high male to female ratio. All those similarities are believed to be a reflection of the similar social and cultural behaviour in those countries (Traboulsi et al. 2006).

89 HIV patients in the period from 1984 to 2008 were managed and received their care at the American University of Beirut Medical Centre (AUBMC) in Lebanon. The average age for HIV patients was 35.4 years and male to female ratio was 6.4:1. Cerebral

toxoplasmosis was the most commonly diagnosed opportunistic infection (21%) reflecting late presentation of patients to medical service. There are few other clinical data about the OIs and AIDS defining illnesses in Lebanon. The lack of studies about the epidemiological profile of AIDS patients in Lebanon makes it difficult to implement effective clinical strategies and programs (Naba et al. 2010). Data concerning HIV/AIDS in Lebanon are limited and this is to be expected considering the political instability with the civil war in the past 10-15 years.

1.2.2.6 Oman

Oman had a cumulative report of 1,539 cases of HIV between 1986 and 1999. Most of these cases were males, and almost half were infected through heterosexual contact (Scrimgeour, Mehta & Suleiman 1999).

More recent data published in 2014 by Oman Ministry of Health (country progress) reported that 2394 people have been diagnosed with HIV infection up to the end of 2013. (Country Progress Report Oman 2014) 115 people were diagnosed in 2013. The most common routes of infection were heterosexual (50.6%), homosexual-bisexual (13.8%), Mother to child (5.7%), IDU (3.8%), blood transfusion (3%) and unknown mode of transmission (23.1%).

Balkhair et al studied the different opportunistic infections in a cohort of hospital admissions of HIV/AIDS patients in Muscat, Oman. This study was published in 2012 and collected data retrospectively from 1998 to 2008 and included 77 HIV/AIDS patients. The results showed that 58% (45) of the patients had one or more OI. The commonest was infection with *Pneumocystis jirovecii* pneumonia (25%), followed by cryptococcal meningitis (22%), cytomegalovirus (CMV) retinitis (17%), disseminated tuberculosis (15%) and cerebral toxoplasmosis (12.5%). This study concluded that there is wide range of OIs in Oman and subsequently in the Gulf region and that the most common OI in Oman were *P. jirovecii* pneumonia and cryptococcal meningitis. (Balkhair et al. 2012)

Apart from Saudi Arabia, Oman is the only country from which there is a report on resistance to ARV's. This study was performed in patients heavily exposed to ARV's and failing regimens and showed high resistance rates (Al Dhahry, Scrimgeour et al. 2004). This is discussed in more detail in Chapter 5.

1.2.2.7 Palestine (Israel)

Between 1985 and 2002, Chemtob and Srour collected epidemiological data for Arabs in Palestine (Israeli Arabs) and they found notifications of 80 patients with HIV/AIDS. The cumulative prevalence rate of HIV/AIDS was 10.1 per 100,000 population (Chemtob & Srour 2005).The study showed that prevalence in Arab Israelis was less than in non-Arab Israelis and neighbouring Arab countries. However, the modes of transmission remained similar to the rest of the Middle East and surrounding countries (Chemtob & Grossman 2004).

For the period of 20 years (1980-2000) retrospective data were collected for 2886 patients with HIV. The overall prevalence of infection was 61/100 000. More than half were males (65.2%) with an average age of 35 years for males and 31 years for females. As in other countries in the region, heterosexual transmission was the most common route, accounting for 68% of cases. Men who have sex with men (MSM) was the second most common mode of transmission in Israel at 18.4% and injecting drug users (IDU) were 8.7%. About 0.6% of HIV/AIDS patients were infected secondary to blood transfusion or after organ transplantation (Chemtob & Grossman 2004).

In 2011 the WHO key data on the HIV epidemic in Israel showed that the prevalence of HIV was 0.2%, with 8500 PLWHIV. At that time, 10.2% of HIV cases were in IDU and MSM accounted for 36.7% (WHO 2011). In 2013 the prevalence of TB coinfection was reported to be 5.8% among HIV positive Israeli patients (Mor 2013).

A more recent review of HIV over the 30 years from 1981-2010 emphasized that 41.3% of all 6579 HIV/AIDS cases in the period were in immigrants from high prevalence countries such as Ethiopia and this group accounted for most of the heterosexual transmission (Zohar et al. 2014). The proportion of MSM in HIV positive men rose from 20.4% in 2000 to 50.9% a decade later. Overall, 13.4% of all cases were related to IDU (Zohar et al. 2014)

These publications emphasize the differences in epidemiological patterns in immigrants, Israeli Arabs and other Israelis. Israel is the only country to have consistently reported on the rapidly increasing HIV related to MSM, and slow but significant increase of IDU use as a risk factor for HIV.

1.2.2.8 Syria

Qualitative interviews were done by Seal and his colleagues in 21 HIV/STI service providers in Damascus and Aleppo in Syria (Seal 2013) . They aimed to evaluate HIV/STI related education, testing, and treatment and to obtain clinical care information. This study also explored the possible barriers to treatment and good medical care for HIV patients. The study aimed to reduce these barriers especially for HIV/STI high risk groups in Syria.

Interviews were performed in early 2011. Most of the physicians questioned thought that HIV patients suffered from more stigma than other STI patients. The physicians said that they rarely asked about a patient's sexual behaviour in detail to avoid embarrassment (Seal 2013).

Therefore, improvement of physician knowledge and attitude towards HIV/STI patients was of key importance. Treatment protocols and standard treatment and management care should be provided. In addition privacy and avoiding patient stigma are essential components of HIV/STI care. There is a need for increased collaboration between HIV and STI services. No clinical descriptions or epidemiological studies about HIV/AIDS were found in Syria apart from the UNAIDS reports which estimate HIV/AIDS prevalence of 0.1% (Seal 2013).

1.2.2.9 Turkey

According to the Turkish Ministry of Health in 2012 there were 5137 HIV cases in Turkey. Most were between ages 20-59, the majority were males (72%) and MSM accounted for 8.9% of cases. Heterosexual transmission is the most common route, accounting for 48% of all HIV cases in Turkey (Agacfidan 2014)

In a Turkish pre marriage programme Alim et al. performed a cross-sectional study of 1,332 pre marriage samples in 2005. The results showed 0.1% to be HIV positive. The study did not specify the difference between the gender, age or mode of transmission (Alim et al. 2009). In 2013 a study was done by Aylikçi, et al to assess knowledge about HIV/AIDS in two high schools in Kırıkkale, Turkey. Most of the 473 students (92.2%) had some knowledge about HIV/AIDS prior to the study. 27% believed that there is a cure for AIDS and 64% believed that HIV can be avoided by using condoms during sexual contact (Aylikçi et al. 2013).

This study reflects the importance of improving young people's knowledge as a key component of HIV/AIDS prevention programmes and destroys the social negative effect on these strategies especially in developing countries.

Table 1-6 summarised the common route of transmission in Turkey from 1985 to 2011 (Mutlu 2014)

Years	1985-1996	1997-2001	2002-2006	2007-2011
Route of transmission	N (%)	N (%)	N (%)	N (%)
Heterosexual	254 (41.2%)	421 (59.5%)	668 (54.8%)	1410 (52.6%)
MSM	65 (10.5%)	40 (5.6%)	105(8.6%)	240 (9%)
IDU	71 (11.5%)	26 (3.7%)	25 (2.1%)	27 (1%)
Blood transfusion	44 (7.1%)	6 (0.8%)	4 (0.3%)	14 (0.5%)

(Table 1-6) The common routes of transmission in Turkey from 1985 to 2011 (Mutlu 2014)

1.2.2.10 United Arab Emirates

As in neighbouring countries, the prevalence of HIV in United Arab Emirates (UAE) is estimated to be low. Al-Dabal et al reviewed the pattern of pulmonary infections in 92 HIV positive persons in Dubai over the 2 years January 2009 to January 2011. They found that 42.2% patients had active pulmonary TB. 77.7% of them had CD4 counts < 200/mm^3 but no patient was receiving ART. 31.1% of reviewed patients had community acquired pneumonia (CAP) and 8.8% had *Pneumocystis jirovecii* .

This study showed that pulmonary TB was most frequently diagnosed illness followed by CAP (Al-Dabal et al. 2011). This study included only one city of the UAE and only a few patients were reviewed. There are

insufficient epidemiological or clinical reviews published from UAE; another report suggested only 1% of HIV patients had TB (UNAIDS 2014)

1.2.2.11 Yemen

UNAIDS has estimated the adult prevalence of HIV in Yemen to be about 0.1%, with the number of HIV/AIDS infected patients increasing from 4000 patients to 12000 in 2004 (UNAIDS 2004). The WHO suggested that a hidden epidemic was on-going in Yemen at that time. The male to female ratio changed considerably in a few years, as in 1995 the male to female ratio for HIV/AIDS infected patients was 4:1, changing to 2:1 in 1999 and 1:1 in 2000 (UNAIDS 2004).

The most common mode of transmission in Yemen was found to be heterosexual in about 77% of all cases, followed by homosexual transmission (16%), blood products (6.8%), and IDU in about 2% (Lambert 2007).

In 2014 Mirzazadeh et al published a study of high risk HIV MSM. The epidemic has not been well explored among this population in most Arab countries. 261 HIV positive men with a history of having sex with men in the previous 6 months from different cities of Yemen were included.

20% (95 % CI 15.8–25.0) reported condom use and 31.4 % (95 % CI 25.9–37.3) reported that they or their sexual partner had a sexually transmitted disease symptom. Injecting drug use was only reported by 0.8 % (95 % CI 0.1–9.2) of this population (Mirzazadeh et al. 2014).

This is an important study concerning very sensitive issues. These could not have been tackled or discussed before in such a conservative society and reflects a very important improvement in overcoming the social barriers in the Region.

1.2.3.1 Saudi Arabia

The first case of HIV in Saudi Arabia was diagnosed in 1984 (Ellis et al. 1993). However, 20 years later, there was still no reasonable estimate of the real statistical and epidemiological state of HIV infections in the country and there were few peer reviewed publications. Only two countries in the MENA region did not provide estimates of the number of HIV/AIDS patients to the Joint United Nations Programme on HIV/AIDS and the WHO Regional Office of the Eastern Mediterranean: these were the Kingdom of Saudi Arabia (KSA) and Afghanistan (Alrajhi 2004). This was because of the nature and the different social understanding of the disease. It is difficult in such a conservative society to address the real HIV/AIDS situation, which is wrongly directly linked to illegal sexual behaviour.

Furthermore, In the UNAIDS/WHO Epidemiological Fact Sheet on KSA 2002 update, none of the parameters for HIV epidemiology could be estimated (Alrajhi 2004). However, the situation has changed continuously over the past decade with improved surveillance, discussion about HIV and publication of data about HIV in the Kingdom.

A more comprehensive review of cases in KSA up to 2001 was published by Madani et al in 2004 (Madani, Al-Mazrou et al. 2004) and showed that heterosexual mode of transmission accounted for 40% of the cases. However, no route of transmission could be identified for 42% of cases (Madani 2004). The study also showed that HIV cases were continuously rising in the Kingdom (figure 1-4).

Figure (1-4) HIV/AIDS cases in KSA between 1984 and 2001 (Madani 2004)

Al-Mazrou et al. published a study of the epidemiological data on HIV/AIDS available in Saudi Arabia in 2003 (Al-Mazrou, Al-Jeffri et al. 2005). They found that there had been 1743 HIV positive Saudi patients and 6064 non Saudi HIV positive patients identified in the Kingdom. Moreover, 872 (50%) of the Saudi HIV patients had AIDS and 77% were males, with a male to female ratio of 3:1. Most of the cases (about 67%) were reported in Jeddah, Riyadh and Dammam. No recent cases were reported to be infected through infected blood transfusion. 46% of the cases were infected through sexual routes.

Finally, the report concluded that Saudi HIV/AIDS cases were increasing significantly every year. The three year survival rate for

AIDS patients had increased by more than 100% from 27% in 1984 to 72% in 2000 and the availability of antiretroviral therapy was assumed to have played a major role in that improvement (Al-Mazrou et al. 2005).

At that time, a survey of 20423 Saudi blood donors in the Central region of KSA showed no cases of HIV, while hepatitis B (HBV) prevalence was 1.5% and hepatitis C (HCV) 0.4% (El-Hazmi 2004). Subsequently, A-Jabri et al showed that rates had increased slightly in pregnant women by 2010. Tests in 11553 pregnant women showed a HIV prevalence of 0.13%, with 1.5 positive cases in 100 000 per year (Al-Jabri et al. 2010).

There were 505 newly diagnosed cases of HIV in Saudi Arabia in 2008, a 34.6% annual increase from previous year. The Jeddah region had the highest proportion of HIV cases in the country (37%) (Madani 2004).

Since 2001 the Saudi Ministry of Health (MOH) has published an annual report specifically for HIV/AIDS in the country. This report included good quality statistics and data about HIV/AIDS in the Kingdom. By 2009, the official MOH reports indicated there have been a total of 3538 HIV/AIDS cases in Saudi (Alothman, Altalhi et al. 2010). In 2012 a retrospective study concerning HIV notifications at Saudi MOH for the ten years (2000 to 2009) was performed. This review showed that a total of new HIV cases of 10217 of which 2958

(29%) were Saudi nationals (Kabbash, Felemban et al. 2012). The discrepancy from the 3538 patients described by Al-Othman et al 2 years previously emphasized problems with data consistency in the Kingdom. The incidence was 1.5 cases per 100 000 for Saudi national patients. Case notifications were increasing yearly until 2009 when they levelled off (Figure 1-6).

In addition the male to female ratio was 4.4:1 in Saudi HIV patients. The study concluded that even though HIV/AIDS reported cases are stabilised from 2006, HIV/AIDS was still considered one of the challenging and important public health problems in KSA (Kabbash, Felemban et al. 2012).

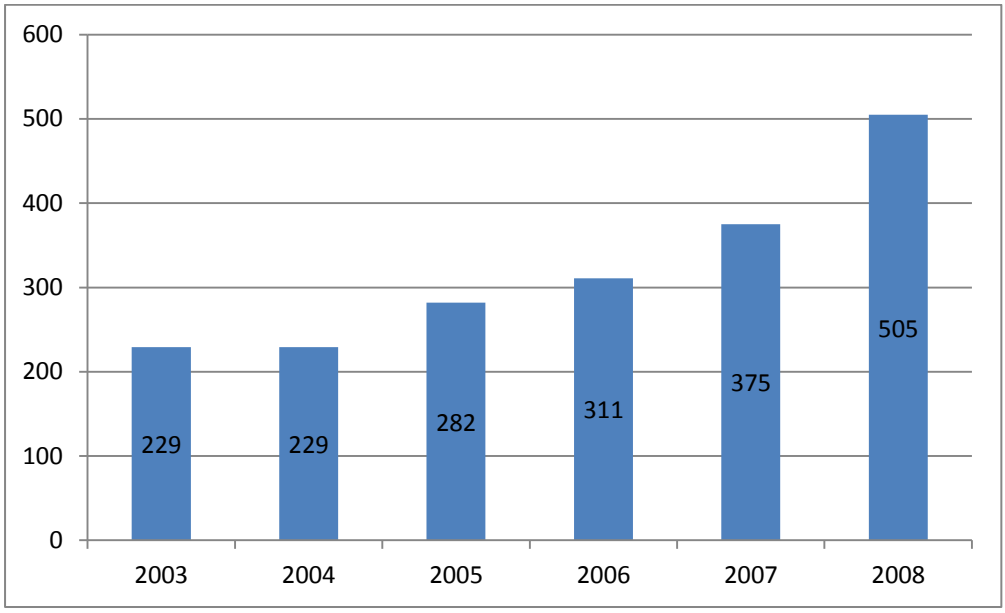


Figure (1-5) Saudi HIV cases annual reports through 2003 -2008 (Kabbash et al. 2012)

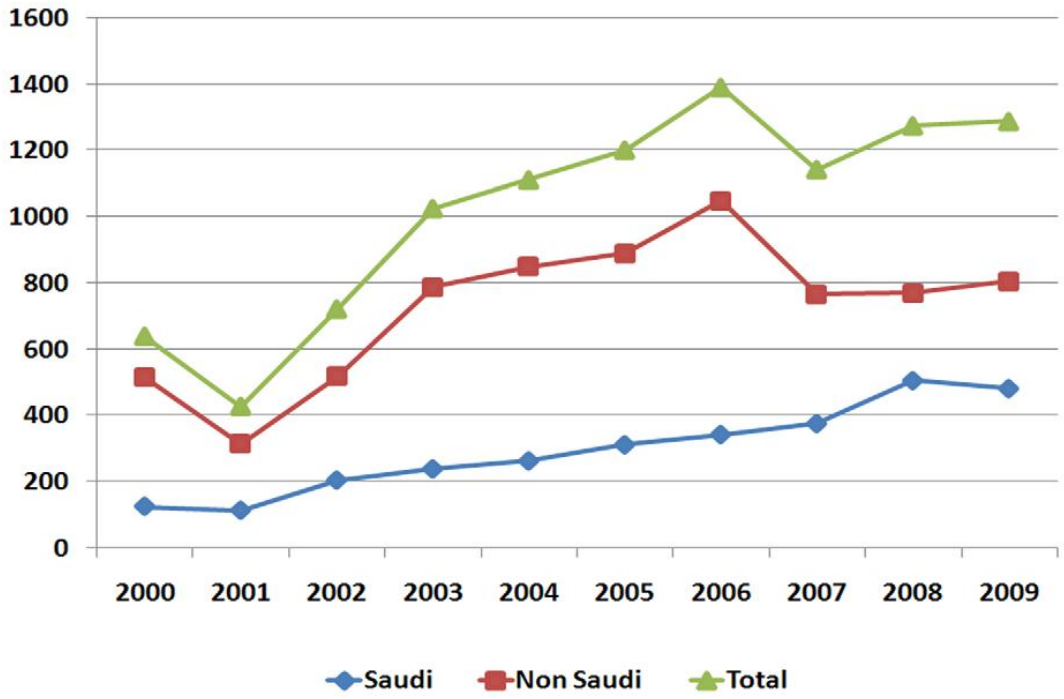


Figure (1-6) Increasing annual HIV/AIDS cases in KSA (Kabbash et al. 2012)

According to the most recent case report statistics from the National Programme for HIV/AIDS Control, there had been 12000 HIV positive people diagnosed in the Kingdom. Almost half of all cases have been found in Jeddah, the main Saudi west coast city (NAP 2014).

The National Saudi AIDS Programme (NAP) is one of the most effective programmes under the General Administration of Infectious Diseases Control, unit of Deputy of Public Health, Ministry of Health, Kingdom of Saudi Arabia. It was established in 1994 and included plans to modernise approved treatment centres after around 10 years, using the latest international guidelines and recommendations for HIV

treatment with ART. The programme supports HIV prevention, care and treatment (NAP 2014).

The NAP is multi-sectorial and has strategies to help in preventing HIV infection and supporting HIV patients around the Kingdom. The NAP is aiming to prevent HIV in Saudi society, especially in those at high risk in order to maintain a low prevalence of HIV in the Kingdom. In addition, the NAP aims to improve the quality of life for HIV/AIDS patients. One of the important roles of the Saudi NAP is to ensure easy access to antiretroviral therapy and comprehensive HIV treatment programmes with appropriate care (NAP 2014).

At the end of 2011, the Ministry of Health (MOH) and the National AIDS Programme (NAP) developed a five-year National Strategic HIV/AIDS Plan (NSP) for the period of 2013-2017.

NAP launched its website (http://www.napksa.com/) and developed a mobile application to disseminate HIV/AIDS related information.

In Saudi Arabia HIV surveillance is integrated into the national communicable disease surveillance system and the national monitoring and evaluation framework. Modes of transmission analysis and data triangulation have not been carried out. There is a national protocol for HIV case reporting. In 2011, 394 HIV new cases were reported and 81.2% of these were in men (WHO 2013).

An HIV test is not routinely offered to pregnant women, and there is no mandatory testing of pregnant women. However, tuberculosis and STI patients are routinely tested for HIV without asking for permission.

In 2012, the national AIDS programme reported that facility based surveys would be conducted in pregnant women, prisoners, STI and tuberculosis patients and in injecting drug users in 2013. Female sex workers and men who have sex with men remain "non-applicable groups" in the official country data for HIV surveillance in Saudi Arabia (WHO 2013).

Since the eighties when the first HIV case was reported in Saudi Arabia, HIV/AIDS has become an important issue from a medical point of view and even more so as a social issue. For many years just talking about HIV has generally considered been an extremely embarrassing, due to the assumption of a direct link with immoral sexual relations. This assumption prevents many individuals from admitting that they are infected and therefore, patients may avoid seeking medical care.

Fortunately, there has been more openness and better understanding in the last 5 years and Saudi society has begun to change these old

barriers. In addition, there is now full support from the government which provides free antiretroviral therapy.

Since the eighties when HIV infection was reported in KSA, there have been few systematic clinical descriptions of HIV cases were insufficient. Only two studies had been published in the whole region on the use of antiretroviral therapy (ART) and the emergence of ART resistance, in Oman in 2004 (Al Dhahry, Scrimgeour et al. 2004) and in Saudi Arabia in 2010 (Jamjoom, Azhar et al. 2010).

1.2.3.2 HIV clinic protocols

HIV services are often provided for persons who have challenges because of factors such as discrimination, poverty or IDU. The context of HIV care still is one of persistent stigma regarding HIV infection itself and discrimination against racial, ethnic, and sexual minorities who constitute the groups with the highest HIV prevalence and incidence. Because of these circumstances HIV clinics should follow certain guidelines to ensure the best possible practice and best use of resources.

For example in the UK, National Institute for Health and Care Excellence (NICE) guidelines and British HIV Association (BHIVA) guidelines recommend that anyone testing HIV positive is seen by a specialist at the earliest possible opportunity, ideally within 48 hours

and at the most within 2 weeks of diagnosis. Similar guidelines are produced by International AIDS group and European AIDS Clinical Society. Diagnosed individuals are usually referred and seen at the HIV clinic in Jeddah within 2-3 weeks in order to join the national AIDS programme. People living with HIV have the right to expect that their care is provided in a safe environment and that everyone is treated with no discrimination.

NICE and BHIVA guidelines also recommend that HIV tests should be routinely requested in GUM, antenatal, drug dependency, TB, viral hepatitis B/C and lymphoma services and to all patients at high risk groups. People attending health care services (primary, secondary and tertiary care) should be offered diagnostic tests for HIV. In Saudi Arabia HIV tests are usually requested in these clinics but this is not yet uniformly followed.

According to Western HIV clinical care guidelines (CDC, NICE, BHIVA), all individuals diagnosed with HIV should be offered a full assessment and treatment at the earliest possibility. In addition they should have their infection monitored regularly and treated safely in accordance with national guidance, and be able to access a comprehensive range of required services.

Essential investigations should be routinely used in the care of people living with HIV; including CD4, lymphocyte counts, HIV viral load measurement and HIV genotype analysis, and results should be

available within 2 weeks of specimen collection. All these essential investigations are routinely done at the HIV clinic in Jeddah for all newly diagnosed HIV individuals apart from HIV genotypic analysis. However, this can be specially requested by the treating physician.

However, when this study started, there was no clear operational clinic guideline about best practice in selecting what test to perform routinely in new patients, such as syphilis, hepatitis and other serology and there was no standard proforma for note keeping. Antiretroviral therapy (ART) and treatment for opportunistic infections are offered free of charge through HIV centres in the Kingdom. Regular follow up services are offered, including clinical follow-up and monitoring of CD4 counts and HIV viral load testing. In HIV-1 infected adults and adolescents, ART is now initiated when the CD4 cell count falls below 500 cells/mm^3. The North American Department of Health and Human Services (DHSS) guidelines are now followed for treatment, available at: http://www.aidsinfo.nih.gov/contentfiles/lvguidelines/adultandadolescentgl.pdf (NAP 2014).

However, ART resistance tests are not routinely done. There is evidence that between 20% and 40% of people receiving ARVs in Europe and the USA are at risk of clinically significant drug interactions (BHIVA 2013). However, there are no data or national

guidelines available concerning assessment or monitoring drug interactions in patients receiving ART in the Kingdom, or in the whole Region.

It has been shown that people living with HIV experience significantly higher rates of depression (BHIVA 2013). The Saudi National AIDS Programme follows guidelines of HIV care in the West, providing continuous emotional and mental health care including psychiatric consultations for HIV patients as required.

A healthy sexual lifestyle is strongly recommended for HIV patients and all patients in the national AIDS programme are educated about sexual health for themselves and their partners, including education about protection of themselves (and others) from acquiring new sexually transmitted infections. In addition they are offered support from qualified staff regarding their sexual contacts and partner notification.

The quality of life for HIV Saudi patients under the national AIDS programme has been improved through economic support and help in finding employment. Health education, treatment and counselling services are given to all HIV individuals within the programme. The NAP has helped to minimize stigma and discrimination of HIV individuals in Saudi society (NAP 2014).

Even though the National AIDS programme in Saudi Arabia is playing an important role in maintaining high quality standard for HIV clinics,

specific operational protocols and guidelines for HIV care and improvement of HIV clinics audit should be developed and used ensure the optimal effectiveness of HIV clinics in the kingdom.

1.2.3.3 Links between Islam and HIV prevalence

Many authors believe that there is a strong link between low HIV/AIDS prevalence in Middle East countries and the religion of Islam (figure 2-6), as Islam prohibits homosexuality and also prohibits extra marital sexual activities. In addition, Islamic rules and values support HIV/AIDS preventive strategies such as prohibiting injecting drug use (IDU) and controlling sexual activities. Moreover, marriage encouraging programmes among youth are of much value in controlling sexual behaviour and for promoting safe sex. Therefore, those strategies were easy to implement in the society as they already believe in the same concept in Islam. But the real practice of these Islamic principles and values are extremely dependant on individual persons and commitment to these Islamic rules varies from one person to another (Gray 2004).

However, some believe that HIV/AIDS cases are generally under reported and that, as reporting systems improve, the data will reveal a higher prevalence of HIV/AIDS in Islamic countries than is currently apparent.

Research about the relationship between HIV and religion is limited because of sensitivity of this subject in all societies. Muula et al in 2012 compared different religious background and HIV prevalence in Malawi, where Christianity is the main religion and Islam is the second. They found no differences among Protestants and Catholics, Muslims, and all Christians combined or between Catholics compared with individual religions (Muula et al 2012).

The situation is skewed in the most countries in the Middle East, where the majority of newly diagnosed HIV individuals are foreign workers (Madani 2004; Gray 2004).
Such individuals are usually deported immediately after diagnosis, which reduces the overall reported incidence and prevalence in these Muslim societies. Muslim countries in the Middle East are in urgent need of improved HIV/AIDS surveillance and more enhanced epidemiological studies in collaboration with national and international prevention programmes (Hasnain 2005; Abu-Raddad et al 2010; Akhtar and Mhamed 2012).

Six out of seven studies have shown a strong relation between a low HIV/AIDS prevalence and Islam. Those studies were done in Sub–Saharan Africa Muslim countries (Gray 2004).

One possible reason for this link is the requirement for circumcision, which is more common in Muslims, in addition to the ritual washing which may play a rule in decreasing transmission. Circumcision has been shown to reduce the risk of HIV transmission (Gray 2004). However, in some Islamic countries, advice not to use condoms by some religious authorities has had a negative effect not only on HIV/AIDS but on transmission of all sexually transmitted diseases in the region (Gray 2004).

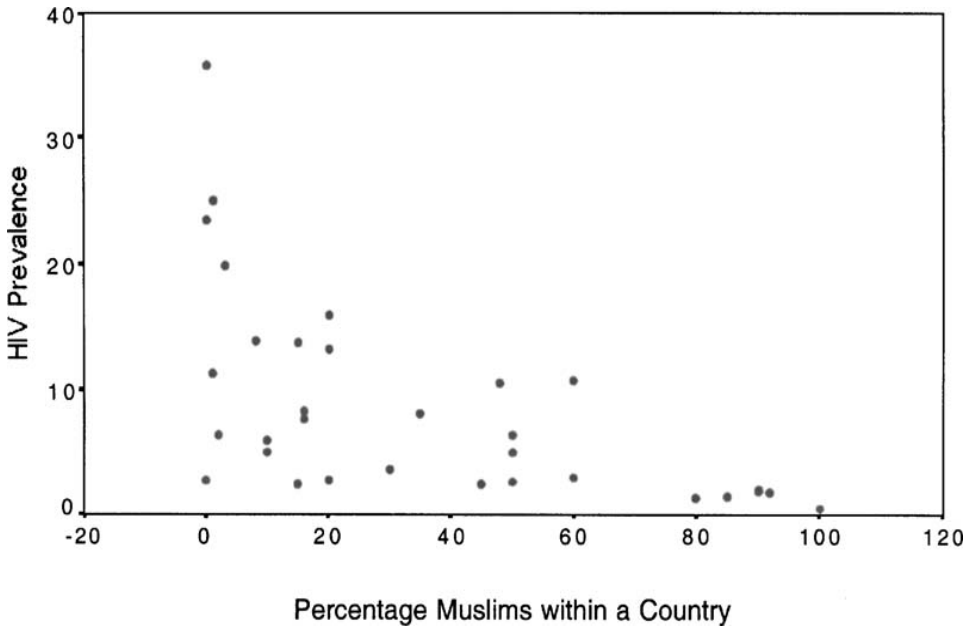


Figure (1-7) HIV/AIDS prevalence in relation to Islam (Gray 2004)

Sexual actions outside marriage are greatly offensive in the Muslim societies, even more than illicit drug use. This can lead to severe discrimination against HIV positive Muslim individuals.

Discrimination starts with the surrounding family and close contacts of the patient and may lead to dramatic isolation of the HIV positive person. In turn, this can deter individuals from notifying themselves to medical authorities and getting necessary medical attention. In addition, it is increasingly recognized that men who have sex with men represent an important hidden source of new HIV infections in several countries in the Region (Shawky, Soliman et al 2009, Mumtaz et al. 2011).

In most societies in the Middle East region including Saudi Arabia, it is commonly known that there is a significant power imbalance between males and females. Females are usually economically dependent on males and in some extreme cases they are not aware of their legal and sexual rights. Therefore, women are more likely to lose their chance of having equal health care than men and are less likely to be tested for HIV. This is may explain why females in such societies are more likely to get infected with HIV without being notified and to have a lower access to health care than males.

There are many especially challenging factors affecting HIV/AIDS transmission and prevention in countries in the MENA region. These include poverty, poor education, wars, social conflicts, refugees, influence of religious leaders, and decreased levels of standard medical care and poor health information.

Further efforts will be needed at both individual and community levels to improve prevention plans and decrease the rising prevalence of HIV/AIDS in Muslim countries (Hasnain 2005). Despite efforts in some politically and economically stable countries in the region, more is needed to overcome these serious barriers. This, includes more health education, particularly sex education, which remains one of the hardest topics to discuss in the region.

To overcome the barriers in prevention plans in such countries, Muslims must recognize there is a big difference between the ideal concepts of Islam which may help in HIV/AIDS prevention, and the situation in real life which really depends on individual practice and behaviours. In addition, full cooperation between religious scholars and public health educators should strive to develop successful and effective prevention plans that are acceptable to society in Muslim countries (Abu-Raddad et al 2010).

1.3. HIV Coinfections:

This section will review the general epidemiology of hepatitis C (HCV), Hepatitis B (HBV), TB and STI infections in the Middle East and in the Kingdom of Saudi Arabia.

There are few data about coinfection with HIV in the whole Middle East Region, illustrated by summary data in Table 1-7 below.

Country	**Year**	HBV Coinfection	**HCV Coinfection**	**TB Coinfection**	**Reference**
Bahrain		NA	NA	NA	
Iran	2009 2011 2013	36.3% (2011)	78% (2009)	10.7% (2013) 11.4% (2011)	Davarpanah et al 2009; Alipour et al 2013 SeyedAlinaghi et al. 2011
Iraq	2006	NA	66% (haemophilia HIV patients)	NA	Al-Kubaisy et al 2006
Israel	2013	NA	NA	5.8%	Mor et al 2013
Kuwait		NA	NA	NA	
Oman	2012	NA	NA	15%	Balkhair et al 2012
Qatar		NA	NA	NA	
Saudi Arabia	2014	3% (Alhuraiji et al 2013)	12% (Alhuraiji et al 2013)	2.16%	UNGASS Country Progress Report 2014
UAE	2014	NA	NA	1% (only 85 cases were tested)	UNGASS country progress report 2014

Table (1-7) Prevalence of HBV, HCV or TB in in patients with HIV in the Middle East (various sources)

1.3.1 Syphilis and STI in Saudi Arabia:

In a conservative country like Saudi Arabia, it is extremely hard to discuss sexually related infections. Therefore, the data are usually of poor quality.

In 1952 a combined national and international venereal disease team started activities in Saudi Arabia. In the Asir region in the south, 309/3000 patients were diagnosed with syphilis. In Abha they noticed a variation of syphilis prevalence among different tribes and different villages but positivity was more common in females than males (El Ghoroury 1954)

A subsequent survey showed that in Saudi Arabia there are two different groups at risk of syphilis infection. These are the nomadic Bedouins who have a high incidence of endemic non-venereal syphilis (bejel), and the people born and bred in towns who were no longer acquiring endemic syphilis but may develop venereal syphilis. This study suggested that bejel accounted for most positive serological reactions found in nomadic communities and probably outweighed venereal syphilis in the population as a whole. The most common late manifestation noted was painful osteoperiostitis of the legs (Pace & Csonka 1984)

These infections are important and increase the likelihood of HIV transmission, especially ulcer casing infections such as syphilis and HSV.

Sexually transmitted infections (STIs) are usually hard to follow and many of infected patients are asymptomatic and diagnosis is therefore missed. Furthermore, if these diseases had been diagnosed they remain unreported especially in developing countries. A total of 39049 STIs were reported to the Ministry of Health (MOH) between 1995 and 1999. These included nongonococcal urethritis (14557 infections, 37.3%), trichomoniasis (10967 infections, 28.1%), gonococcal urethritis (5547 infections, 14.2%), syphilis (3385 infections, 8.7%), HIV (2917 infections, 7.5%), genital warts (1382, 3.5%), genital herpes (216 infections, 0.6%), and chancroid (78 infections, 0.2%). Nongonococcal urethritis, trichomoniasis, and gonococcal urethritis were the most commonly reported STIs in Saudi Arabia (Madani 2006).

Fageeh conducted a study on patients infected with genital herpes simplex (HSV) at King Abdulaziz University Hospital in Jeddah, Saudi Arabia in the period 2003-2011. 343 patients were included in this study and 13.1% were HIV coinfected. 12.5% of the HSV infected patients were coinfected with chlamydia and 12.8% were also infected with gonorrhoea. Genital ulcer disease was diagnosed in

57.9% and HBV serology (HBsAg) was positive in 2.3% (Fageeh 2013).

In 2014 a more recent study reported on the sexual behaviour and knowledge about HIV/AIDS and sexually transmitted infections (STI) in women inmates of Briman Prison, Jeddah, Saudi Arabia. 204 women aged 16-60 years (mean, 33.3 years) were interviewed. Overall, 83% were not aware of STI and HIV infections. 57% had not been screened for HIV or STI before sexual intercourse or marriage. 42.6% of the interviewed women were not sure about the importance and the role of using condoms in protection from infections. Among the 204 interviewed women, only 10 (4.9%) were using condoms during sexual intercourse to protect themselves from possible infections. The study concluded that efforts should be made to overcome this poor knowledge about STI, HIV infection and risky sexual behaviours (Fageeh et al. 2014).

1.3.2 Hepatitis A (HAV):

Hepatitis A (HAV) is transmitted by oro-faecal routes and infection in childhood used to be universal in most parts of the tropics and also in the Middle East including Saudi Arabia (Asghar 2014). The incidence of HAV is increased in some groups at risk of HIV including men who have sex with men and in Western settings, such risk groups are recommended to be immunised (Crum-Cianflone et al. 2010). It is

mostly a self-limiting disease but it may cause serious liver injury which can leads to fulminant hepatic failure and death. The WHO estimates that HAV infection causes nearly 1.4 million new cases worldwide yearly (Asghar 2014).

Similarly, it has been estimated that HAV serology prevalence in Saudi Arabia was 18.6%, a considerable reduction from the 90-100% rates reported only two decades ago in the adult population. A serial comparative study of HAV antibody positive patients in Saudi adults between 1989 and 2008 found a significant decrease from 53% in 1989 to 25% in 1997, and finally to 18.6% in 2008 (Alhetheel et al 2014).

The epidemiology of hepatitis A has changed in much of the Middle East, partly due to improvement of water supply and in other countries due to immunization (Bawazir et al. 2010). In neighbouring Yemen, the prevalence of positive hepatitis A serology in adults was 86% in 2010. The improvement in prevalence of HAV infection from the past in Aden could be related to the quality of water supply (Bawazir et al. 2010).

1.3.3. Hepatitis B Viral Infection (HBV):

About one third of the world's population have been infected with hepatitis B. Most of the cases occur in the tropics and in the Middle East. Saudi Arabia was one of the first countries to including hepatitis B vaccine in the national vaccine programme and recent data show that Saudi Arabia is now classified by the WHO as an intermediate endemic region for hepatitis B (Andre 2000).

Hepatitis B is a DNA virus. It has direct oncogenic effects as well as those due to chronic hepatitis in carriers, leading to clinically significant chronic liver disease, liver failure and liver cancer in about 25% of carriers (Bawazir et al. 2010; El-Serag 2012). It is one of leading causes of the preventable cancer in the tropics, now being reduced due to global effects to vaccinate children (El-Serag 2012)

The virus is transmitted from mother to infant at birth, to children in early childhood by unknown reasons, probably mostly related to unsafe injections (Kermode 2004), blood transfusion and sex. In most non Western settings, including the Middle East, transmission in infancy and childhood is the most important mode of transmission. However, presence of HIV coinfection in the mother probably enhances perinatal HBV transmission.

Adults with HIV are at increased risk of HBV due to similar patterns of transmission (Chang et al. 2014). While high risk HIV populations in Western settings have higher risks of HBV exposure, the situation is less clear in tropics. In Sub Saharan and West Africa, prevalence of HBsAg carriage varies from intermediate (2-7%) and high (8% or more)(WHO 2012).

The serological picture of HBV co infection in HIV/AIDS patient may be different from HBV infection in other individuals. For management purposes it is important to establish a diagnosis of chronic HBV infection in HIV patients, as some antiretroviral drugs (lamivudine, emtricitabine, tenofovir) also have activity against HBV. HIV/HBV coinfected patient are likely to develop drug resistant mutations of HBV if given ART regimens containing only one agent effective against HBV, so ART regimens for coinfected patient usually contain at least two ARVs which also act against HBV, typically tenofovir and emtricitabine (BHIVA 2013). As with hepatitis C, patients with HIV/HBV coinfecton may have a hepatic "flare" 2-3 months after starting ARV's, due to immune reconstitution inflammatory response (IRIS) (Chang et al. 2014).

Identification of HBV coinfection is, thus, important for patient management and non-immune HIV positive individuals should be vaccinated against HBV (Kottilil, Jackson & Polis 2005).

HBV is used to be a highly endemic in the Kingdom in the late 1980's with a prevalence of up to 7% for HBsAg and more than 70% for the presence of any hepatitis marker. In 1989 the Saudi government started a compulsory vaccination programme to all children and healthcare workers. The latest statistics from blood donors showed that the expected prevalence of HBV (HBsAg) ranges from 1.5% to 2.6% in the adult Saudi population (Abdo et al 2012).

No data have been available until recently for HBV coinfection in HIV positive individuals in Saudi Arabia, but data from some neighbouring countries are summarised in Table (1-7). Data from Sub-Saharan Africa are available from a few studies. In general most of those studies in Sub-Saharan Africa showed an increase of about two folds or less, whereas in non-African countries most of the studies showed an increase of at least four fold for HBV co infection in HIV/AIDS patients (Burnett et al. 2005). More recent studies in Malawi show only a slight increase in HBV infection in HIV positive individuals compared to non HIV positives (Chasela et al. 2014), whereas in Ghana, where there is a high background of prevalence of HBV carriage serology, the risks were greater in HIV positive patients (Geretti et al. 2010).The only study of HBV coinfection in HIV patient individuals in Saudi Arabia does not show substantially high rates of HBV coinfection, being present in 3%

of a cohort reviewed from January 1985 to December 2010 (Alhuraiji et al. 2013).

1.3.4. Hepatitis C Viral Infection (HCV):

The WHO estimates that 3% of the global population are currently infected with hepatitis C (HCV) with 3-4 million patients newly infected every year, and that HCV infection is equating to >185 million infections worldwide (Messina et al. 2015) . About 80% of chronic liver disease (CLD) is secondary to infection with hepatitis C. In studies of 653 CLD patients in the Western region of Saudi Arabia, 35.4% of Saudi patients were positive for HCV antibody. Moreover, 39.5% of 223 patients with hepatoma were HCV antibody positive (Akbar 2004).

Despite the importance of HCV infection there were poor data on the prevalence or incidence of the infection in the KSA.

The Saudi blood donation protocol is strict in providing HCV antibody testing for all donors. In 1997, 2.7% of blood donors were infected with HCV (Akbar 2004) and more than 500,000 Saudis are already HCV positive. A subsequent study in 2002 showed different rates of HCV in Saudi blood donors in different regions of the country as follows: the Western region was the most affected region with a prevalence of 1%, then the Southern region at 0.9%, then Eastern region with a prevalence of 0.6%, the Northern region with

prevalence of 0.7% and the lowest prevalence was 0.4% in the Central region (Akbar 2004).

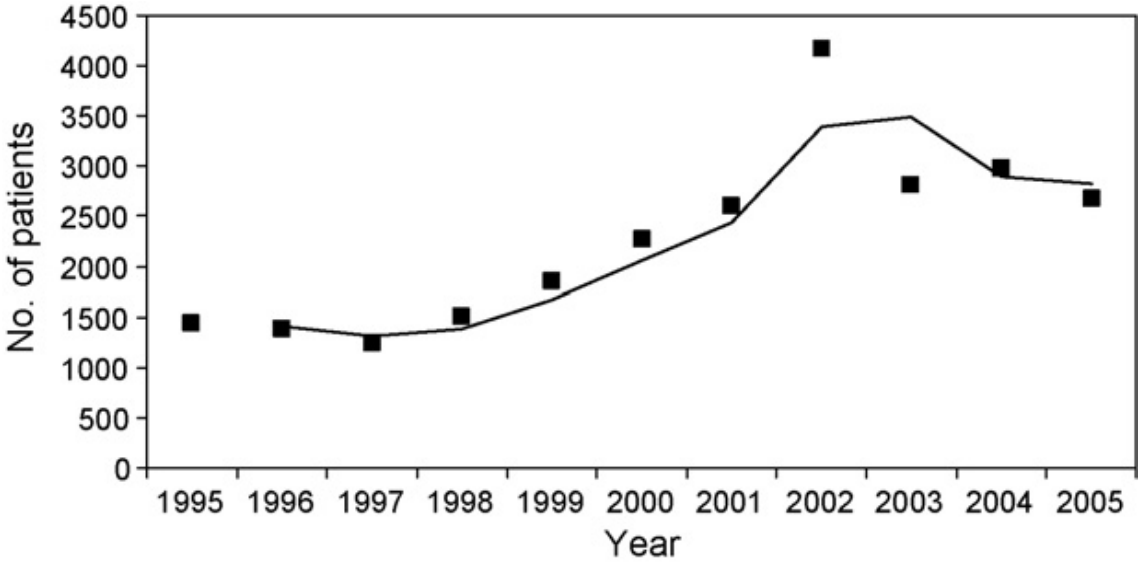


Figure (1-8) Annually reported hepatitis C virus cases in Saudi Arabia (1995-2005) (Madani 2009).

Conversely, HCV prevalence was later calculated in each Saudi administrative provinces and found to be highest in Al Baha and Jeddah (32%) and the lowest in Jizan (1.6%) during the period 1995 to 2006 (Abdo et al 2012).

The prevalence of HCV infection in most neighbouring and Islamic countries was similar to that in Saudi Arabia but ranges from 0.2% in Algeria to 7.9% in Libya. Table (1-8) shows the different prevalence rates in each country.

No.	Country	HCV Prevalence
1	Algeria	0.2%
2	Egypt	18.1%
3	Indonesia	2.1%
4	Iraq	0.5%
5	Jordan	2.1%
6	Kuwait	3.3%
7	Libya	7.9%
8	Malaysia	3.0%
9	Mauritania	1.1%
10	Morocco	1.1%
11	Oman	0.9%
12	Pakistan	2.4%
13	Palestine	2.2%
14	Qatar	2.8%
15	Somalia	0.9%
16	Sudan	3.2%
17	Tunisia	0.7%
18	Turkey	1.5%
19	United Arab Emirates	0.8%
20	Yemen	2.6%

Table (1-8) HCV prevalence in some Islamic and Neighbouring countries around Saudi Arabia (Madani 2009)

The prevalence of HCV in Saudi Arabia is markedly higher in adult age groups than the children (figure 3-2). In older adults, this is may be related to past blood transfusions, and in younger adults substance injecting misuse plays a major role in both HCV and HIV transmission (Madani 2009).

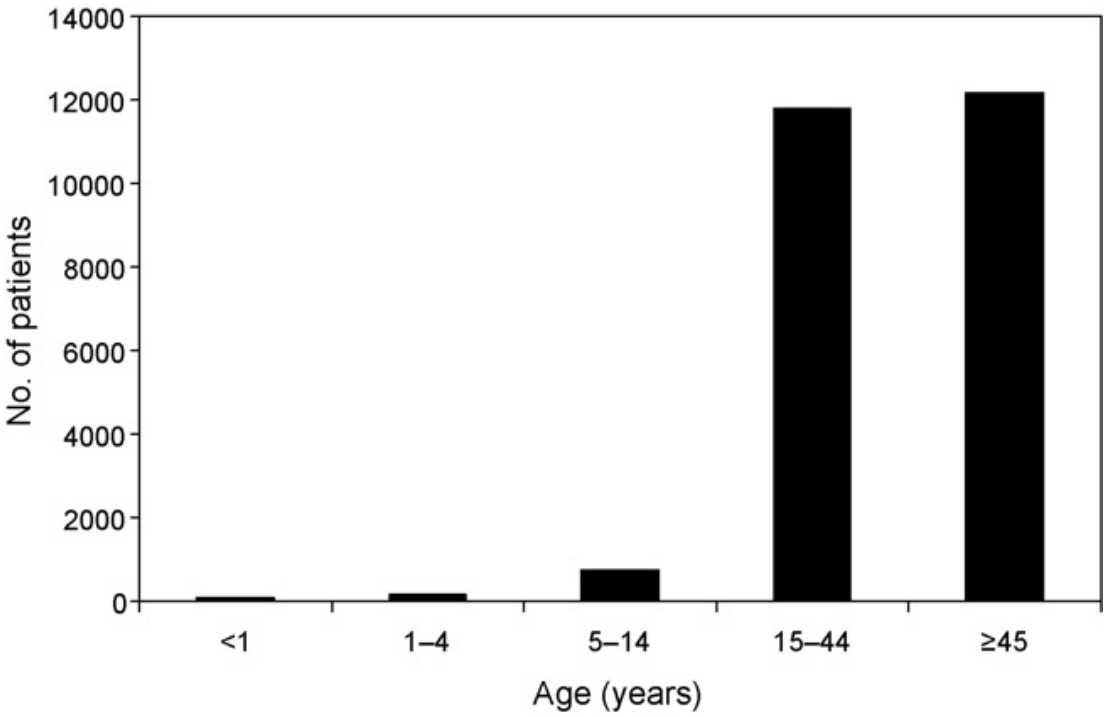


Figure (1-9) HCV infection according to age group in Saudi Arabia (1995—2005) (Madani 2009)

Transmission by blood and blood products are common transmission routes for both HCV and HIV. Alzahrani et al. examined detection of HCV and HIV seropositivity rates in expatriates in Dammam city in the Eastern region of Saudi Arabia in 2009. This study included 875 non-Saudi citizens who were tested for HCV antibodies, showing two positive and two equivocal results. On testing for HCV RNA and also using a HCV antigen-antibody combination assay the equivocal results were confirmed to be positive ie 4/875 samples were positive. Only one sample was positive for HIV. The authors concluded that addition of antigen detection assays to the screening of HCV and HIV may improve detection and lowering transmission for both viruses in Saudi Arabia (Alzahrani et al. 2009).

HCV co infection is one of the important and emerging infections which may have a serious effect on HIV/AIDS patients and will lead to increase of mortality and morbidity. In particular HIV coinfection greatly accelerates the prognosis of hepatotoxicity and fibrosis and cirrhosis due to HCV (Kottilil, Jackson & Polis 2005).

HCV coinfection is now considered to be an indication for starting ART in HIV positive individuals, irrespective of CD4 count or clinical staging (BHIVA 2013).

There may be drug interactions between antiretrovirals especially protease inhibitors, pattern of various therapies for HIV may interacts with directly acting agents for HCV (Karageorgopoulos et al. 2014)

Until recently, no epidemiological data have been available about coinfection of HCV and HIV in the Kingdom of Saudi Arabia. There are two series of studies in the Region considering HCV coinfection in HIV patients. In Iran, Davarpanah et al in 2009 found that the prevalence of HCV infection in HIV positive injecting drug users was 78% (Davarpanah et al 2009). In Iraq a study of haemophiliac HIV positive patients in Baghdad found that 66% were coinfected with HCV.

In late 2013 a study looked at HIV patients in Saudi Arabia and coinfections with HCV or HBV. This study collected data from HIV/AIDS patients who had been followed at King Faisal Specialist Hospital in Riyadh between 1985 and 2010. Coinfection with HCV was found in 12% of the cases. 88% of those were haemophiliac patients or receiving blood or blood products for other reasons (Alhuraiji et al 2013).

In the same cohort, hepatitis B was found in 3% of patients. The most common risk factor for HBV infection among HIV patients in this study was heterosexual transmission in 73% patients (Alhuraiji et al 2013).

Summary of hepatitis (A, B and C) serology interpretations are listed in Table (1-9)

Hepatitis A Antibody (ant-HAV IgG or IgM)	Screen for immunity to hepatitis A; vaccinate those not immune	Negative	Offer hepatitis A vaccine if indicated
		Positive	IgG: Immun, no vaccine necessary IgM : Acute infection
Hepatitis B Surface Antigen (HBsAg)	Indicates active hepatitis B infection	negative HBsAg	Most likely, no chronic infection (may be falsely negative) Vaccinate if anti-HBcAb and anti-HBsAb negative (not immune)
		positive HBsAg	Indicates chronic or acute hepatitis B infection; requires further evaluation (check HBV DNA)
Hepatitis B Surface Antibody (Anti-HBs)	Indicates immunity status, due to past infection or immunization	negative anti-HBs	The patient is not immune to hepatitis B; consider vaccination, unless patient has active hepatitis (HBsAg positive or HBV DNA positive)
		positive anti-HBs	The patient is immune to hepatitis B either by previous infection or by immunization; may be negative in acute hepatitis B infection
Hepatitis B Core Antibody (Anti-HBc IgG)	Indicates past infection or ongoing infection Not affected by immunization	negative Anti-HBc	The patient most likely has not been infected with hepatitis B; consider vaccination if anti-HBsAb negative and anti-HBsAg negative
		positive Anti-HBc	The patient most likely has been infected with hepatitis B; this test alone does not distinguish past exposure and active infection In rare cases, may be falsely negative in some patients with chronic infection, although positive anti HB-IgM is significant in acute or very active chronic infection If anti-HBsAb negative and sAg negative check HBV DNA to rule out active infection; vaccinate if HBV DNA is not detected. If anti-HBsAb is positive, patient is immune
Hepatitis Be antigen HBeAg and	Determining infectivity of hepatitis B virus (HBV) carriers and monitoring infection status of chronically infected patients	positive HBeAg	Presence of hepatitis Be (HBe) antigen and absence of HBe antibody usually indicate active hepatitis B virus (HBV) replication and high infectivity.
		negative HBeAg	Absence of HBe antigen with appearance of HBe antibody is consistent with loss of HBV infectivity.
Hepatitis Be antibody anti –HbeAb		positive anti-HBeAb	Absence of HBe antigen with appearance of HBe antibody is consistent with reduced of HBV infectivity.
		negative anti-HBeAb	Although resolution of chronic HBV infection generally follows appearance of HBe antibody, the HBV carrier state may persist.
HBV DNA PCR	Indicate level of virus infection	Negative HBV DNA PCR	Negative or weakly positive (below detection limit)
		Positive HBV DNA PCR	High levels positive in present of HBeAg carrier with high infectious state and progression to chronic liver disease
Hepatitis C Antibody (HCV IgG)	Hepatitis C	negative anti-HCV Ab	Patient is not infected with hepatitis C Screen at baseline; consider annual screening for high-risk patients, and if clinically indicated. May be false negative in HIV patient (check PCR in this situation)
		positive anti-HCV Ab	Patient has chronic hepatitis C infection or past infection with spontaneous clearance (no protective immunity); confirm positive results with HCV RNA.
HCV PCR	Detection of acute hepatitis C virus, confirmation of chronic HCV and quantification of HCV	Undetected	HCV is absent in the patient's serum specimen
		Detected	HCV RNA level indicates HCV viral replication in the patient. It is important to assess response to therapy.

Table (1-9) Interpolation of hepatitis (A,B and C) virus serology

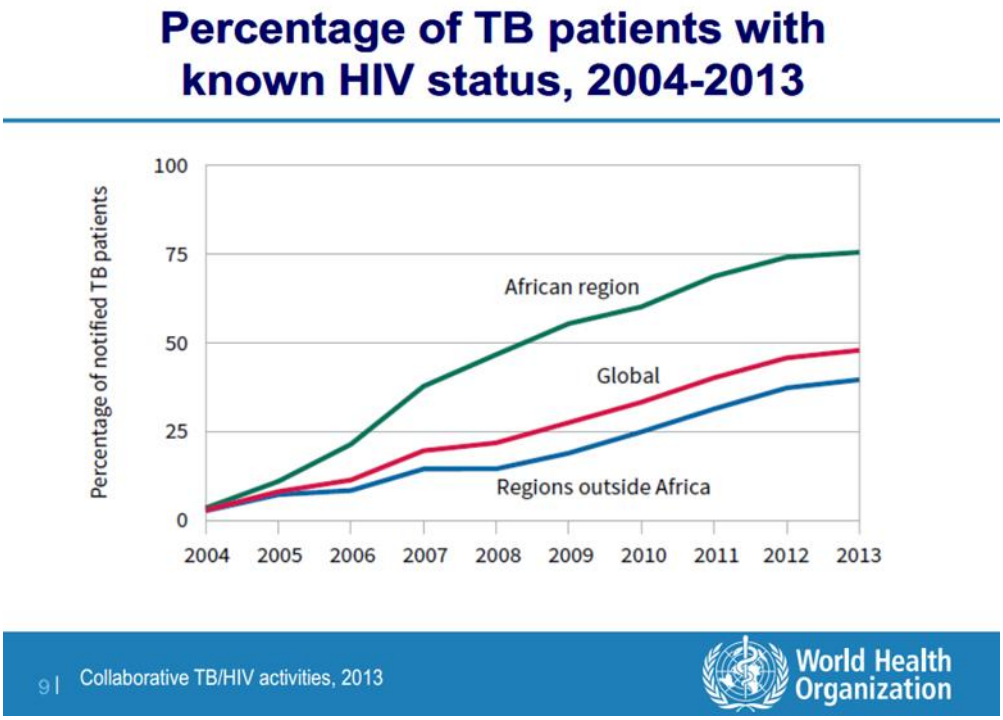
1.3.5. Tuberculosis Infection (TB):

Tuberculosis (TB) remains one of the most important global health problems. This importance has increased dramatically in association with the HIV epidemiology especially in low and middle income countries (Tanimura et al. 2014).

Tuberculosis is considered to be the most common co infection in HIV positive individuals and one of the leading causes of mortality in HIV/AIDS patients in high TB endemic countries. The WHO estimated that about 13% of HIV/AIDS cases died from TB coinfection (WHO 2007).

About 9.3 million new TB cases were reported in 2007, of which about 15% (1.4 million) were newly HIV diagnosed patients. There were 1.7 million deaths due to TB were, of which 0.46 million were HIV positive patients (WHO 2009).

TB was a major problem in countries in Africa even before the HIV/AIDS endemic and escalated in the late 1990's with HIV. In some African countries, more than three quarters of TB notifications were in HIV positive patients (WHO 2013). Similar but less dramatic associations are seen in other regions (Figure 1-10).



(Figure 1-10) Percentage of TB patients with HIV (WHO 2013)

Most TB coinfections with HIV are due to reactivation of latent TB, and HIV positive people who have latent TB are about 50 times more likely to have active TB coinfection (Antonucci et al. 1995). Therefore, the incidence of TB infection in HIV positive people is significantly higher than the general population (Antonucci et al. 1995; El-Sadr & Tsiouris 2008). However, it is now recognised that HIV positive individuals in highly endemic regions for TB, such as Sub-Saharan Africa, are very susceptible to both reactivation or new infection and thus guidelines recommending prolonged chemoprophylaxis against TB in HIV positve patients in this setting (Golub et al. 2015)

This has gained further impact with the emergence of multidrug resistance and extreme drug resistance in TB in parallel with HIV. Rapid TB diagnostics and TB drug susceptibility testing for all patients remain a priority in this region (Post et al. 2014)

In the Middle East and Eastern Mediterranean region the WHO estimated in 2009 that 20,517 (about 1.5% of global cases) new cases of HIV/TB coinfection are found in this region, but the impact of HIV on increasing TB infection was not thought be very significant. However, in the same report, 30.5% of TB cases in the Eastern Mediterranean region were found in HIV/AIDS patients (Zarocostas 2009).

In Israel TB coinfection was found in 5.8% of HIV positive patients in 2013 (Mor 2013). Iran had TB coinfection rates of 10.7% in 2013 (Alipour et al 2013). Balkhair et al in 2012 showed that about 15% of HIV Omani patients were coinfected with TB (Balkhair et al 2012).

Unfortunately the Saudi Ministry of Health yearly report did not show the TB reported cases among HIV/AIDS patients until recently. A study done in 2010 by Omair et al showed that the incidence of TB coinfection in HIV positive individuals is at least 30 times higher than it is in the general Saudi population. This study concluded that the incidence of TB coinfection in HIV/AIDS patients in Saudi Arabia is

significantly higher than in the general Saudi population and that mortality is also high despite early diagnosis and treatment (Omair, Al-Ghamdi & Alrajhi 2010).

In the latest Saudi Arabian UNGASS country progress report in 2014 the Saudi Ministry of Health (MOH) reported that 2.16% of HIV Saudi patients are coinfected with TB (UNGASS Country Progress Report 2014). Until recently there were no specific guidelines in the Kingdom about tuberculosis testing or isoniazid prophylaxis against TB for HIV positive individuals attending services of TB patients for HIV infection.

Case notification for TB has improved over the past two decates in Saudi Arabia, along with treatment and TB control strategies. In comparison to neighbouring countries Saudi Arabia has a moderate rate of TB infection (Al-Hajoj 2009) (Table 1-10). This situation might be expected in a large country with around 6 million expatriates mostly coming from countries where TB is more endemic, and the annual pilgrimage (Hajj) season, when millions of multinationals come to the holy Muslim places.

Infection rates in Saudi Arabia are different from region to region. Jeddah, the west coast and the main port to pilgrimage (Hajj) has the highest infection rate with 64 per 100,000 cases. In comparison in Riyadh, the capital and central region of Saudi Arabia, the infection rate is 32 per 100,000 cases (Al-Hajoj 2009).

#	Country	Infection rates	Reference
1	USA	5.2 per 100,000	(Broekmans et al. 2002)
2	UK	35 per 100,000	(Broekmans et al. 2002)
3	Saudi Arabia	32—64 per 100,000	(Maguire et al. 2002)
4	India	180 per 100,000	(Porco et al. 2006)
5	Sub-Saharan Africa	290 per 100,000	(Corbett et al. 2003)
6	South Africa	509 per 100,000	(Corbett, Watt et al. 2003)

Table (1-10) TB Infection rate in Saudi Arabia compared with other countries (Al-Hajoj 2009).

Data collected in a retrospective study in King Abdulaziz Hospital, Jeddah over 3 years from medical records showed that from a total of 157 cases diagnosed with pulmonary TB, 36% were Saudi patients and the majority (64%) were non Saudi patients. The mean age was 33 years (+/- 15.3). This study suggested that the majority of TB cases in Saudi Arabia especially those cases in Western region (Jeddah) are including imported by visitors to the Kingdom (Al-Hajoj 2009).

TB infection rates of 60 per 100,000 were reported by the Saudi Ministry of Health in 2007 and total number of TB cases has increased slightly from 3854 cases in 2006 to 3878 cases in 2007 (Al-Hajoj 2009). Data from the same study found that Saudi TB patient was 52.7% while non-Saudi TB cases were 47.3%. This changing picture reflects the urgent need for better plans and strategies to fight and control TB in Saudi Arabia, and emphasised the need for reporting and furthermore, frequent studies on TB in Saudi Arabia (Al-Hajoj 2009). In addition, collaboration between local and international health organizations to provide the best management and control for TB patient in the country was highly recommended (Al-Hajoj 2009).

Saudi Arabia remains a moderate TB burden country with an incidence rate of 18/100,000 (Al-Hajoj & Varghese 2015).

Few studies have been conducted to explore the real situation of first line TB treatment resistance and MDR-TB in the Kingdom, with various results ranging from 14% to 20% for the first line treatment and from 1% to 44% for MDR-TB. Until 2013, the true burden of drug-resistant TB was unknown, as there were no representative national surveys to measure levels and patterns of anti-TB drug resistance. In 2013, Al-Hajoj et al. reported the results of 1902 TB patients and showed first line treatment resistance of 23% and MDR-TB of 4 % (Al-Hajoj & Varghese 2015)

1.3.6 IDU and HIV infection

Injecting drug use is one of the most important risk factors for HIV transmission in many countries, but the prevalence of IDU among HIV positive individuals varies from one region to another. Even within a same country, there are marked differences in estimated HIV prevalence. Countries in Southeast Asia are of particular concern, as are Eastern Europe and Latin America, where HIV prevalence is estimated to be over 40% in IDU. Only 8 countries out of 148 reported zero prevalence of HIV patients in IDU populations (Mathers et al. 2008)

The transmission of HIV and other blood borne viruses leads to increases the significant morbidity and mortality associated with drug misuse and the life style of many injecting drug users reduces their access to success of ART (Vallecillo et al. 2014). In the last 10 years, reporting of IDU and HIV rates in this population has improved. (Mathers et al. 2008)

Previous estimates suggested that worldwide there were around 16 million (range 11–21 million) IDU, and the patchy data then available suggested that about 3 million (range 1–7 million) IDU were living with HIV (Ball, Rana & Dehne 1998). There were few data to support the effectiveness of HIV preventions which targeted IDU in developing countries. However, there is evidence to support HIV prevention

programmes implemented in many other sociocultural settings (Ball, Rana & Dehne 1998).

Malekinejad et al. conducted a cross-sectional survey in Iran to obtain HIV prevalence and risk behaviour in injection drug users in Tehran. HIV prevalence was 26.6% and was higher specifically in IDU who were sharing needles (Malekinejad et al. 2015).

Injecting drug use is illegal in most countries and is particularly difficult to deal with in most Arabic countries. The reported importance of IDU as a risk factor for HIV varies from country to country, and is particularly important in Iran Bahrain and Oman (Malekinejad et al. 2015; Mathers et al. 2008).

In the Kingdom of Saudi Arabia, IDU is probably underestimated for social reasons but the proportion of IDU to HIV has varied from 0.14% in 1996 (Njoh & Zimmo 1997) to 10% in 2010 (Alshomrani 2014). The drug services in Jeddah, Saudi Arabia have been established for many years and in the past have not shown high rates of HIV in IDU.

A study was performed in Al-Amal Hospital in Jeddah in 1996 to determine the prevalence of HIV among drug users, including patients visiting the hospital in 1995 - 1996. Only 3/2101 of the cases were HIV positive and the overall prevalence for HIV in drug users was 0.14% (Njoh & Zimmo 1997)

A more recent study examined of the inpatient records of admitted heroin addict to Al-Amal Hospital in Riyadh, KSA between 2006 and 2009. 247 of 379 inpatients were IDU and included in the study. All the patients were males. The prevalences of HBV, HCV and HIV were 9%, 82% and 10%, respectively. The study concluded that HCV infection is 20 times higher in Saudi heroin addicts than in the general population while HIV infection is more than 600 fold higher, and the infection with HBV was not much different from that in the general population (Alshomrani 2014).

Country	Year	IDU+HIV (n/N)
Australia	2006	1.5% (2243 /149591)
Bahrain	2000	0.3% (NA)
Israel	2006	2.94% (NA)
Italy	1996	12.1% (39120 /326000)
Iran	2015	26% (449/1726)
Kuwait		NA
Oman	2005	11.8% (NA)
Qatar		NA
Saudi Arabia	1997	0.14% (3 /2101)
	2014	10% (24/247) (Alshomrani 2014)
Spain	1998	39.7% (33336/ 83972)
Thailand	2001	42.5% (68224 /160528)
Turkey	2005	2.6% (NA)
UK	2006	2.3% (3597/156398)
USA	2002	8.7% (161589/1857354)

Table (1-11) Prevalence of HIV infection in IDU (Mathers et al. 2008, Malekinejad et al. 2015)

1.3.7 Summary

As in the rest of the Region, Saudi Arabia has an obvious deficit in data about the epidemiology and dedicated clinical information about many features of HIV infection. Coinfections with TB, HCV and/or HBV are important in this patient group but the available data are patchy and not always consistent.

Simultaneously, there are no systematic reviews or data series about the epidemiological and socio-behavioural issues affecting high risk and hard to reach groups of patients, who may pose a particular risk for transmission of HIV in the kingdom.

Therefore, this study aimed to improve these data gaps and increase the quality of data for HIV/AIDS patients in the Kingdom. The primary aims were to provide data on the clinical features, associated with risk factors and operational aspects of patient management in the largest HIV clinic in in the kingdom, in Jeddah.

The aims were:

1. Review the epidemiology of HIV in the Middle East and in particular in the Kingdom of Saudi Arabia (KSA).
2. Review Regional and KSA data on coinfection of HIV with hepatitis B, C and tuberculosis.
3. Describe the clinical presentation, demographic features and rates of coinfection in a large outpatient HIV clinic in Jeddah.
4. Investigate the prevalence and types of primary resistance to antiretroviral therapy in HIV positive patients in Jeddah.

1.4. Antiretroviral Therapy (ART):

The introduction of antiretroviral therapy (ART) and in particular combined highly active antiretroviral therapy (HAART), has dramatically changed the management and prognosis for people live with HIV, such that a normal or near normal life expectancy is predicted (Williams et al. 2014)

There are several different classes of ART, each of which targets a phase of the HIV live cycle. These main classes include (Figure 1-11)

i. Nucleoside Reverse Transcriptase Inhibitors (NRTIs).
ii. Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs).
iii. Protease Inhibitors (PIs).
iv. CCR5 receptor blocker.
v. Integrase inhibitors.

There are many factors which may affect the clinical effectiveness of ART, including the importance of combining drugs from different classes. Adherence to ART is also one of the most important factors in control of infection and prevention of the emergence of resistance to ART.

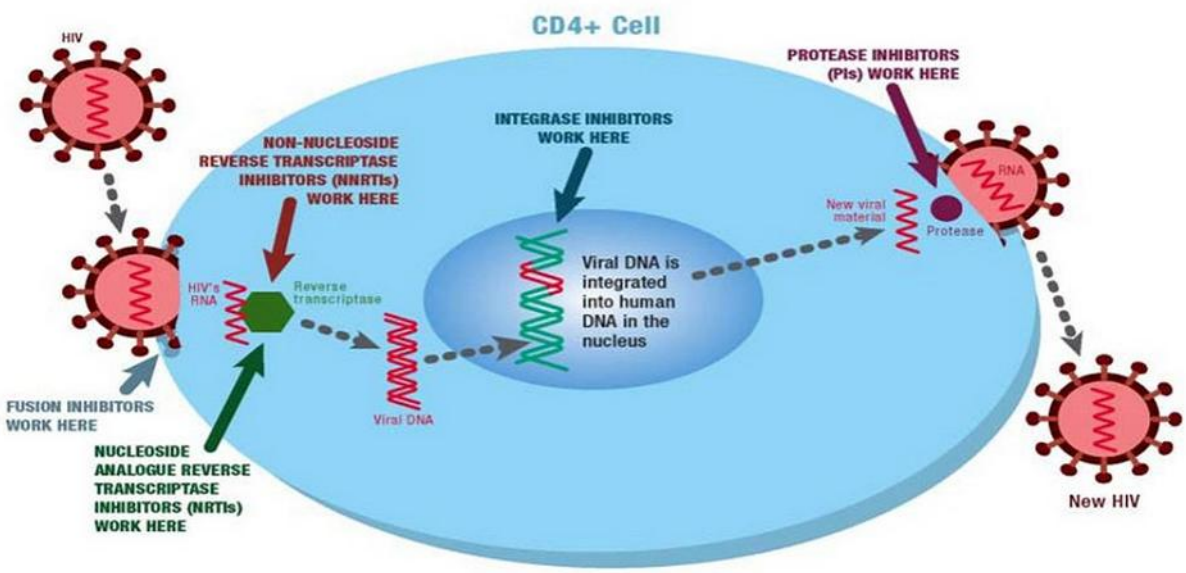


Figure (1-11) ARV and HIV life Cycle (Gebremaryam 2014)

Over time HIV/AIDs treatment has been developed and improved from using a single ARV medication to the use of combinations of several classes of ARV therapy (Fischl, Richman et al. 1987, Enanoria, Ng et al. 2004). The use of HAART has become the standard of care in modern clinical practice and detailed protocols of best practice are available internationally and frequently updated (Williams et al. 2014). The introduction of triple highly active antiretroviral therapy (HAART) has dramatically improved the clinical management of HIV/AIDS patients. Studies performed soon after HAART became available in 1996 showed a

remarkable decline in mortality and morbidity of up to 85% in treated patients (Palella, Delaney et al. 1998, Flepp, Schiffer et al. 2001).

However, about 2 years after the FDA approval of the use of zidovudine (ZDV), the first available NRTI, the first drug resistance was discovered in 1992 (Erice, Mayers et al. 1993). Resistance to ART medication is an important and crucial issue in management of HIV/AIDS patients. Reporting of resistance to ARV's has been increasing since the first discovery in 1992 and gradually included all ART classes (Johnson, Brun-Vezinet et al. 2006).

Further experience has shown that patients must adhere rigorously to the full treatment regimen, with considerable loss of efficacy and the risk of emergence of drug resistance if the medication is taken less than 90% of the time (Romanelli & Pomeroy 2000). Poor adherence is encouraged by the frequent side-effects caused by the medication, logistic difficulties in drug supply or clinic attendance, and more complex psychosocial issues. Interruptions of treatment are clearly associated with increased risk of drug resistance and subsequent failure of treatment (Romanelli & Pomeroy 2000). In resource poor settings, access to antiretroviral therapy is increasing due to the development of extensive international HIV/AIDS organization collaborations as the UNAIDS Estimation and Projection Package (EPP) (Brown et al. 2010).

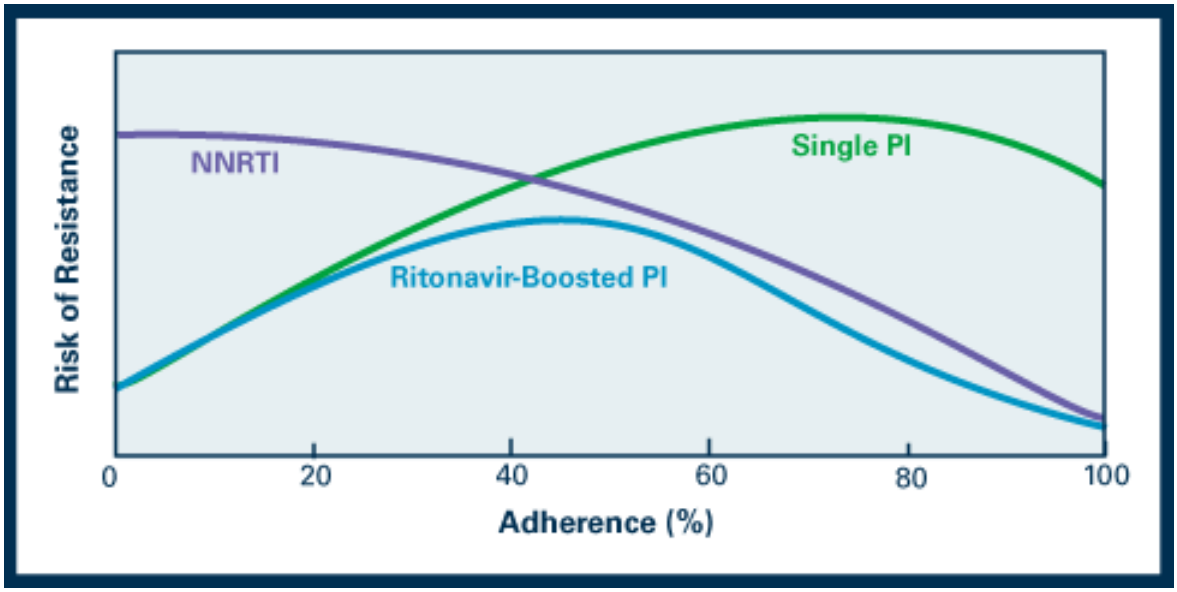


Figure (1-12) Adherence and ART resistance (Bangsberg, Moss & Deeks 2004)

A meta-analysis of the efficacy of ART in HIV/AIDS programmes in resource poor settings concluded that ART programmes in such countries may have similar results and may be just effective as in well developed countries (Ivers, Kendrick & Doucette 2005). However even with these organization and input of international panels such the International AIDS Society-USA, this is not universal and such roll-out programmes are vulnerable to interruptions in drug supplies (Hirsch et al. 2008).

Therefore, it is helpful to have ART sensitivity tests to assess the effectiveness of ART in individuals with HIV infection. This is a crucial management tool to help to determine the best ART combination therapy for patients with HIV and to guide changes if resistance emerges (Johnson, Brun-Vezinet et al. 2006).

1.4.1 Types of ART resistance testing:

When ART resistance testing was first introduced, there were few standard definitions for the role of ARV resistance testing. In clinical practice ARV testing is used to predict the failure of treatment rather than measuring success. There were several ARV resistance assays (genotypic and phenotypic) already in use in the clinical field and these have evolved over the time (Mayers 1996). Currently, the most common form of resistance testing is genotypic resistance testing. Mutations are known to be associated with certain ARV resistance (Lessells, Avalos & de Oliveira 2013).

Resistance usually occurs because HIV has a very rapid replication and HIV reverse transcriptase has no proofreading function. Therefore, mutations may occur even before ART is started. The resistant virus is usually present at low concentrations in the absence of treatment. However, once the patient starts on ART, the low numbers of mutated virus rapidly becomes higher. Very low concentrations of mutated virus population can be missed by either resistance testing method, due to sampling errors in a population of HIV that is predominantly sensitive to ARV's.

There are two main types of measuring ARV resistance. Phenotype and genotype test.

Phenotypic ARV resistance testing is an in vitro measurement of the concentration of ARV drug needed to inhibit 50% of growth (IC50) or 90%

(IC90) of growth of HIV culture in the laboratory (Rodriguez-Rosado, Briones et al. 1999). It is performed by growing the virus in culture media with various amounts of drugs added. This allows direct measure of viral resistance but it does not explore nature of the underlying mutations, just their effect on the ability of the drug to stop the virus from replication. Figure (1-13) summarising the relationship between drug concentration and viral inhibition (Loi, Modlinski & Ptak 2011).

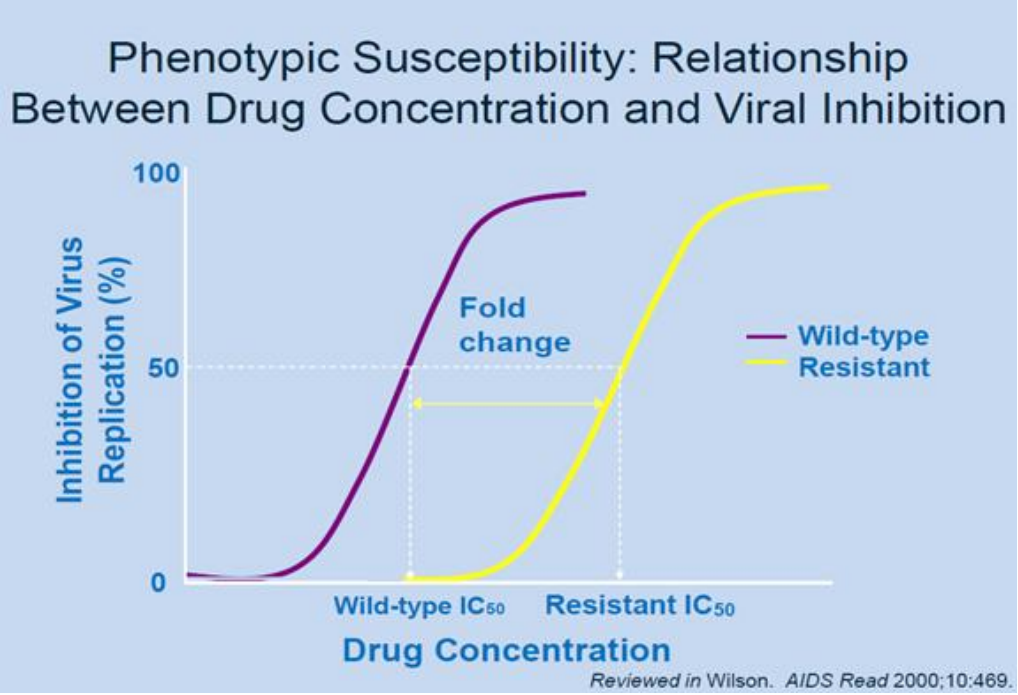


Figure (1-13) Phenotypic Susceptibility: the resistance shifts the inhibition of in vitro HIV growth by ARV to the right (Loi et al. 2011)

Phenotypic resistance testing is easy to interpret, but it is expensive and time consuming with a high degree of variability (2.5 to 4 fold). It cannot identify trends or small changes and does not take into effect pharmacokinetics: some drugs may be clinically ineffective if this is only a 2x change in phenotypic resistance, while for others a 10x change may be needed before a clinically important effect is noted (Perez-Elias et al. 2003).

Virtual phenotypic testing is a way of interpreting genotypic test results. After the sample is tested by genotypic method, phenotypic test results for other virus samples with a similar genotypic pattern are taken from a database. Matched samples inform how the virus is likely to behave. The virtual phenotype is faster and less expensive than a phenotypic test (Perez-Elias et al. 2003).

In 2003 a study by Perez-Elias et al. compared ART choices depending on phenotypic or virtual phenotypic tests, which were compared in a prospective, randomized, double-blind, multicentre, controlled clinical trial. They found that virtual phenotypic testing is at least as effective as phenotypic testing when used to select an optimized treatment for patients who have failed one or more antiretroviral regimens (Perez-Elias et al. 2003).

The virtual phenotype procedure is a computerised method characterized by a simple laboratory experiment that yields complex information on the patient, which is interpretable in terms of the relevant disease phenotype (Lengauer, Pfeifer & Kaiser 2014).

Genotypic resistance refers to the mutations that are selected for by drugs when a patient has virological failure. Genotypic resistance testing looks for the genome sequences in HIV RNA virus that have mutations conferring resistance to specific antiretroviral drugs. This is performed by isolating virus from serum/plasma of the patients and directly sequencing the viral nucleic acid coding for reverse transcriptase and protease. Resistance to specific drugs is predicted based on known point mutations in genetic sequences in each location (Tisdale et al. 1993, Loi et al.2011).

Such tests aim to provide more accurate information at the genome level about mutations that may lead to ART resistance. This helps clinicians as well as patients to have the best ART choice and hence to improve therapy plans and treatment outcomes (MSAC 2009)

Results from viral RNA sequencing are compared to a large database of wild type HIV sequencing. The Stanford HIV Drug Resistance Database hosts a freely available online genotypic resistance interpretation system called HIVdb. This system is available to help clinicians and laboratories interpret HIV-1 genotypic resistance tests including resistance and susceptibility of NRTI, NNRTI and PI. The HIVdb genotypic resistance system gives the results as an XML report (Liu & Shafer 2006) and can be accessed through the website http://hivdb.stanford.edu/pages/webservices/. Examples are shown in (Figure 1-14)

Genotypic resistance testing is relatively fast and inexpensive. However, in relation to the interpretation of results, phenotypic resistance testing is

easier to interpret, and the contribution of some mutations to phenotypic resistance is controversial. Occasionally, certain combinations of mutations can interact to restore ART sensitivity although individually they act to confer resistance (Mayer et al.2001). ARV resistance in the Region is reviewed in chapter 5.

Metzner et al. tested 4 patients who experienced early virological failure of a first-line regimen of lamivudine for ART resistance. Before treatment no drug resistance was noted by the standard genotype analysis. A few weeks after of starting ART, most of the minority quasispecies were rapidly selected and represented the major virus population. The study concluded that minority mutations of drug-resistant viruses, can rapidly become the major virus population and lead to ART resistance in naïve patients when the start treatment (Metzner et al. 2009). This explains the need to monitor HIV viral load after starting treatment, and to test for resistance if viral load suppression is suboptimal (BHIVA 2013)

The current prevalence and patterns of HIV drug-resistance in treatment-naïve and treatment-experienced patients in KSA are unknown. In line with initiatives taken internationally, the National authority in the Kingdom is supporting this study as part of the Saudi policy of supporting medical research to inform national treatment programmes.

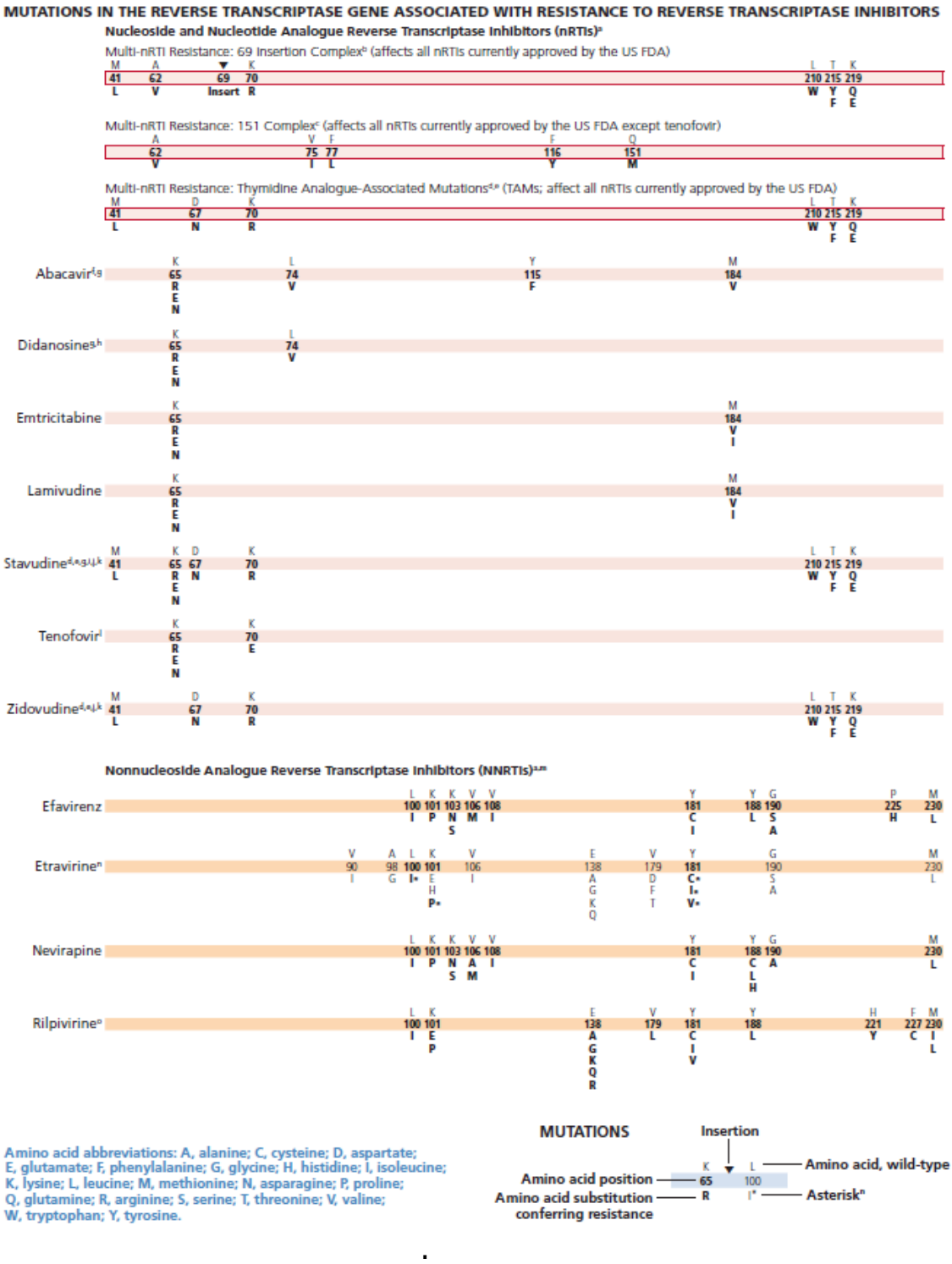


Figure 1-14 Significant mutations IAS-USA (Wensing et al. 2014)

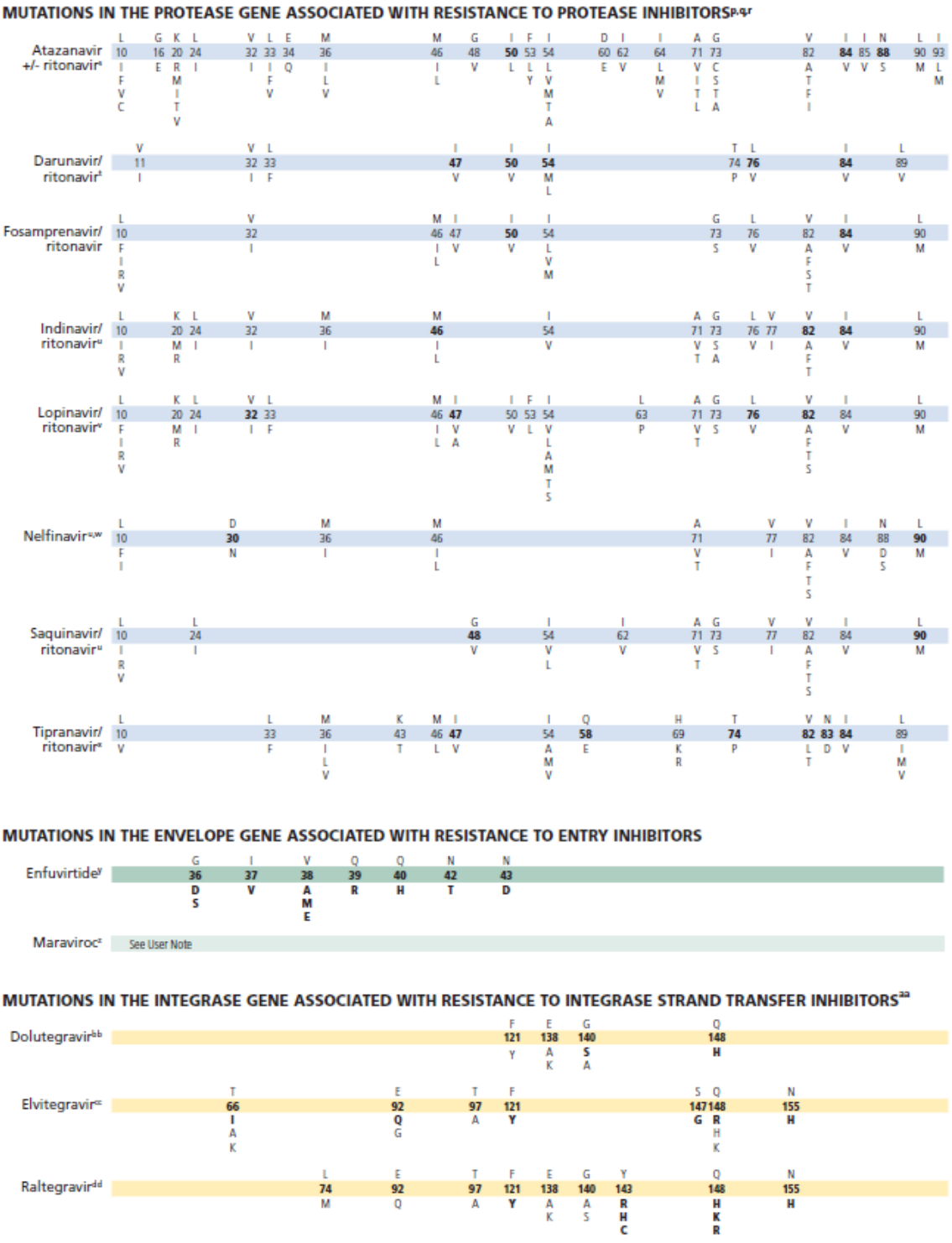


Figure 1-14 Significant mutations IAS-USA (Wensing et al. 2014)

Chapter 2

Methodology

2.1 Clinical and laboratory features of HIV/AIDS in the Kingdom of Saudi Arabia:

This is a descriptive, retrospective cohort study, performed in the HIV outpatient clinics at King Saud Hospital (Infectious Disease Hospital) in Jeddah, KSA.

These clinics are part of the national Saudi programme for HIV patients under Ministry of Health (MOH) supervision. Primary contact was established with the coordinator of the Saudi AIDS national programme in Jeddah region, Dr. Felmban and the Hospital Director General, Dr. Shikhon, for the study plans and methods. Initially it was thought that other hospitals might be included in the study according to availability of patients and data follow up, but this was not logistically possible.

During the planning phase, contact was made with Dr. Mohamed Baksh, general director of King Abdulaziz (Red Sea) Hospital in South Jeddah (MOH), which had up to 120 admissions with HIV a year but relatively little outpatient activity. However, the study finally focused on the clinics of King Saud Hospital North Jeddah (Programme Director, Dr. Sana Filmban), which provide the main clinics for patients in Jeddah in the Saudi programme for HIV prevention.

This hospital has 150 beds with up to 60 admissions a year for HIV and 120 admissions for other infectious diseases. In the year of the study in 2012 there were 3 clinics a week for a cohort of about 800 adults with

HIV, run by 3 doctors (specialist and 2 consultants) and 6 specialist nurses.

Patients are referred from all other clinics and hospitals in Jeddah and ART is provided free from the hospital pharmacy. Patients typically attend every 2-3 months for follow up.

There is no specific community outreach. There was no comprehensive management protocol but usually the same protocols recommended and used by the developed Western countries such as BHIVA guidelines are followed by the treating physician at the clinic. The most commonly prescribed ART is a three drugs regimen containing 2 NRTI (usually tenfovir/emtricitabine or abacavir/lamivudine and formerly zidovudine and a third agent (NNRTI, PI or more recently INI). For treatment naïve patients the most prescribed combination is tenofovir, emtricitabine and efavirenz, with nevirapine as an alternative NNRTI. The usual protease inhibitors at that time included ritonavir boosted lopinavir or darunavir.

Enzyme-linked immunosorbent assay (ELISA) HIV test is the main screening test for Saudi Arabian Ministry of Health which is usually confirmed by Western-Blot (WB) test or more recently, a second ELISA test. A compulsory HIV screening test is required in case of clinical suspicion, preoccupation screening, before marriage and routine HIV blood test for hospitalized patients or those followed in clinics.
HIV tests are compulsory before getting married and before taking up several occupations. This is the rule for all governmental careers and

occupations in the Ministry of Labour in Saudi Arabia. They are also performed routinely on inpatients and patients attending general outpatient clinics. They also may be requested on clinical suspicion of HIV.

All HIV related special tests in Jeddah are centralized. CD4+ counts and HIV viral load are available in King Saud Hospital (Infectious Disease Hospital), King Abdulaziz University Hospital Lab and Jeddah Central Lab by MOH in Jeddah. HIV resistance test and HLA B*5701 test are not routinely available for HIV patients, but can be done at the King Abdulaziz University Hospital Lab. Records of patients with resistance to ART are available in the HIV lab records and individual patient files from which data can been extracted.

All patient data are created in computer and individual clinical records. These records are stored confidentially in the hospital record department with access only to authorized persons. As a registered Saudi doctor I was permitted to attend the clinics and permission was obtained to allow me to access to the medical records department, where the clinical patient records are kept safely in computers and hard drives.

2.2 Data collection and anonymising

A list of all patients who had ever attended the clinic in the years 2000-2010 was provided and I extracted the computerized case records of each patient within the same records area of the hospital.

Required data (detailed in appendix B) included patient's code, age or date of birth, sex, education level and marital status, duration of infection, age at diagnosis. Clinical data from the patient's history in the records included presenting history, how HIV was diagnosed, risk factors and history of coinfections.

Laboratory data included WBC, Hb, lymphocytes and absolute $CD4^+$ cell count. CD4 percentage was not routinely recorded for all patients, however it was available for some patients as was $CD8^+$ cell count. Serology for common viruses and other infections were not available for all patients and I recorded each result as positive, negative or not done. Viral load was also recorded in copies/mm^3

Data required for study were copied manually from the primary source on a standardized paper record form designed for the study (Appendix A). Data were stored anonymously, using a code labelled to specific patients in a log listing designed for the study (Appendix B). Anonymized individual records were transferred into a secure excel spreadsheet on the investigator's laptop and stored as password encrypted data and password protected laptop. No case records or identifiable patient data

left the records area of the hospital or clinic. Access to the anonymized data was for myself and my supervisor only.

2.3.1 Important definitions:

HIV/AIDS patients: this included all HIV positive patients seen at King Saud Hospital (Infectious Disease Hospital) outpatient clinics in the 11 year period 2000-2010 inclusive.

HIV cases were staged according to WHO criteria (WHO 2007)(Table 2-1)

2.3.2 TB co infection:

This followed the World Health Organization (WHO 2007) definition for TB diagnosis depending on sputum smear positive for acid fast bacilli (AFB). This definition has been also confirmed by joint publication of the WHO, the International Union against Tuberculosis and Lung Disease and the Royal Netherlands Tuberculosis association (KNCV) (WHO 2007).

International policy for TB case detection:

1- Tuberculosis in a patient with at least two initial sputum smear examinations positive for acid fast bacillus (AFB+), or
2- Tuberculosis in a patient with one sputum examination positive for AFB+ and radiographic abnormalities consistent with active pulmonary tuberculosis as determined by the treating medical officer, or
3- Tuberculosis in a patient with one sputum specimen positive for AFB+ and culture positive for AFB+.

The revised definition of a new sputum smear-positive pulmonary TB case is based on the presence of at least one acid fast bacillus (AFB+) in at least one sputum sample in countries with a well-functioning external quality assurance (EQA) system.

2.3.3 HCV co infection:

In this study HCV coinfection was defined as HIV patients who have a positive HCV antibody test. Unfortunately there were no HCV PCR tests available in the vast majority of the patients, which was one of the limitations of this study. Interpretation of HCV serology is summarised in table (1-9)

2.3.4 HBV co infection:

In this study HBV coinfection was defined as HIV patients who have positive HBV surface antigen test (HBsAg). There were no anti-HBV antibody tests, which was a limitation of the study. Detailed interpretation of HBV serology is summarised in table (1-9)

2.4.1 WHO AIDS Staging

Clinical Stage	Clinical Conditions or Symptoms
Primary HIV Infection	• Asymptomatic • Acute retroviral syndrome
Clinical Stage 1	• Asymptomatic • Persistent generalized lymphadenopathy
Clinical Stage 2	• Moderate unexplained weight loss (<10% of presumed or measured body weight) • Recurrent respiratory infections (sinusitis, tonsillitis, otitis media, and pharyngitis) • Herpes zoster • Angular cheilitis • Recurrent oral ulceration • Papular pruritic eruptions • Seborrheic dermatitis • Fungal nail infections
Clinical Stage 3	Unexplained severe weight loss (>10% of presumed or measured body weight) • Unexplained chronic diarrhoea for >1 month • Unexplained persistent fever for >1 month (>37.6°C, intermittent or constant) • Persistent oral candidiasis (thrush) • Oral hairy leukoplakia • Pulmonary tuberculosis (current) • Severe presumed bacterial infections (e.g., pneumonia, empyema, pyomyositis, bone or joint infection, meningitis, bacteraemia) • Acute necrotizing ulcerative stomatitis, gingivitis, or periodontitis • Unexplained anaemia (haemoglobin <8 g/dL) • Neutropenia (neutrophils <500 cells/µL) • Chronic thrombocytopenia (platelets <50,000 cells/µL)
Clinical Stage 4	• HIV wasting syndrome • Pneumocystis pneumonia • Recurrent severe bacterial pneumonia • Chronic herpes simplex infection (orolabial, genital, or anorectal site for >1 month or visceral herpes at any site) • Esophageal candidiasis (or candidiasis of trachea, bronchi, or lungs) • Extrapulmonary tuberculosis • Kaposi sarcoma • Cytomegalovirus infection (retinitis or infection of other organs) • Central nervous system toxoplasmosis • HIV encephalopathy • Cryptococcosis, extrapulmonary (including meningitis) • Disseminated no tuberculosis mycobacteria infection • Progressive multifocal leukoencephalopathy • Candida of the trachea, bronchi, or lungs • Chronic cryptosporidiosis (with diarrhoea) • Chronic isosporiasis • Disseminated mycosis (e.g., histoplasmosis, coccidioidomycosis, penicilliosis) • Recurrent nontyphoidal Salmonella bacteremia • Lymphoma (cerebral or B-cell non-Hodgkin) • Invasive cervical carcinoma • Atypical disseminated leishmaniasis • Symptomatic HIV-associated nephropathy • Symptomatic HIV-associated cardiomyopathy • Reactivation of American trypanosomiasis (meningoencephalitis or myocarditis)

Table (2-1) WHO Clinical staging (WHO 2007)

Data were processed and analysed using double entry into Excel (Microsoft office 2010) and SPSS V22 programs (IBM 2013). Statistical analysis included quantitative descriptive analysis and summary statistics (means, median, percentages, standard deviations, etc) with comparisons of demographic and other features.

The sample size required according to the statistical power calculations were as follows:

The expected rate of hepatitis C co-infection was about 3.5%. A sample size of 2000 is thus expected to produce 70 co-infected and 1930 non coinfected HIV positive patients. These numbers would provide 80% power to detect factors potentially related to co-infection with odds ratios (OR) of between 2.1 and 2.6, and 90% power to detect factors potentially related to co-infection with odds ratios of 2.3 to 3.0 (assuming the risk factor is present in 10% to 50% of the non-co-infected group).

The expected rate of TB co-infection was about 7.4%. A sample size of 2000 is thus expected to produce 148 co-infected and 1852 non co-infected HIV positive patients. These numbers will provide 80% power to detect factors potentially related to co-infection with odds ratios of between 1.65 and 2.0, and 90% power to detect factors potentially related to co-infection with odds ratios of 1.8 to 2.2 (assuming the risk factor is present in 10% to 50% of the non-co-infected group).

At the time of data collection the total number of patients found officially registered in the Jeddah clinic was 1383 patients. All these were included in the retrospective study, as a preliminary for more focused prospective data collection in the second study later.

Data collection was performed in 2011. Prior ethical permissions were obtained from the Research Ethics committee of Liverpool School of Tropical Medicine (LSTM) (ref. 10.66) and approved in 6 November 2010. Permission was obtained from the local authorities in KSA (Appendix E).

2.5. Antiretroviral resistance in treatment naïve HIV patients in Jeddah

The study included HIV positive Saudi patients who were attending the clinics or had been admitted to King Saud Hospital in Jeddah. The study has several sequential phases.

These clinics are part of the national Saudi programme for HIV patients under Ministry of Health (MOH) supervision. As described in detail in chapter 5, 1383 patients attended the clinic from 2000 to 2010, of whom 25% had never received ART.

Laboratory work for genotypic resistance testing by Sanger method took place at the Liverpool Virology Centre at the Royal Liverpool University Hospital. I have participated in basic laboratory techniques but advanced techniques and advanced sequencing analysis were done by professional virology lab staff. Samples were then sent to the Public

Health England laboratory in Colindale London, UK for genotypic resistance testing by next generation sequencing (NGS) method.

2.5.1 Patient recruitment

Over the period of 3 months (November 2013 – February 2014) blood samples were collected from HIV treatment naïve cases attending King Saud Hospital in Jeddah. All 664 patients attending in this period were approached and screened for eligibility for inclusion.

Patients attending the clinic routinely provide consent for blood samples to be taken for their routine care. ART resistance testing is not routinely available in KSA, although it is essential for best practice. Testing is currently only available as a special request in KSA.

For this study, patients were asked to provide an extra 10-15 ml of blood as part of their clinical management as well as for research purposes. It was explained that part of the sample would be used primarily for research purposes to inform about patterns of new HIV infections in the Kingdom.

All patients were informed in detail and in their native language about the procedure and the test. The blood sample was taken by a well-trained and authorised medical doctor and the WHO guideline for blood drawing in HIV patients was strictly followed (WHO 2010). Storage and transportation of the samples were performed according to CDC guidelines (CDC 2010).

Participants gave informed written consent (Appendix C) and coded data were recorded at the clinic visit including basic demographic and clinical features and details of ART exposure. These were collected anonymously onto a standardised anonymised data form, using a study code to link the data with the blood sample taken at the visit (Appendix A). Results were coded and the code list was known only to the treating clinician at KSH clinics in Jeddah to ensure confidentiality.

Minor patients (under age of 18 years) and patients who refused to participate were excluded from the study.

Primary contact had been established with the coordinator of the Saudi AIDS national programme, Assistant Deputy Minister for Preventive Medicine and the Hospital Medical Director study plans and methods

The contacts are summarised in the table (2-2) below. While this is an overall description (anonymised) of the whole clinic population, patients who provide clinical data and blood samples had improved medical care, as data on ARV resistance were sent back to the treating physician with code numbers.

Hospital name	Address	Contacted person
Ministry of Health Saudi Arabia	Riyadh (MOH)	Dr.Ziad Memish (Infectious Disease Consultant & Assistant Deputy Minister for Preventive Medicine)
King Saud Hospital	Jeddah (KSH)	Dr.Batool Ali (Infectious Disease Consultant & Hospital Medical Director)

Table (2-2) main Jeddah contacts

2.6 Samples storage and transport

Samples were sent initially to King Saud Hospital Laboratory in 10ml EDTA tubes and processed according to the manufacturer's guidelines. Whole blood was centrifuged and serum and plasma separated within 8 hours of blood withdrawal. The minimum plasma sample volume required is 6 ml, which was shipped in three vials each contain 2ml in addition to serum vial which contain at least 2ml.

Blood samples were collected from all newly diagnosed patients seen during the study period and all patients who were not on ART. The original estimated sample size was 100-150 patients.

Plasma and serum samples were stored at KSH laboratory. Under a Transport Agreement (MTA) and International Air Transport Association (IATA) regulations, collected blood samples were refrigerated and stored in -70 ^{o}C according to CDC guideline for

collection and transportation of human specimen. They were transported to the Liverpool Virology Centre by approved international courier. The samples underwent sequencing of the HIV-1 RNA genome to detect drug resistance and determine the HIV-1 subtype.

2.7. Lab work methodology:

The in-house HIV resistance test in Liverpool was used for partial sequencing of the *pol* region of the HIV genome for detection of anti-retroviral resistance mutations.

2.7.1 Principles of examination:

This is a one-step real time-polymerase chain reaction (RT-PCR) which amplifies a 1284bp segment of the HIV pol region. PCR is then used to generate two overlapping products to cover protease codons and codons 1-235 of the reverse transcriptase gene. These products are sequenced in both directions and interpreted using the Stanford University HIV Drug Resistance Database (SDRD) version 6.31 20th September 2013.

2.7.2 HIV drug-resistance testing/nucleic acid sequencing:

HIV RNA plasma was isolated the by automated extraction, amplification of the viral genetic region of interest by PCR, and subsequently sequencing the amplified product by population (Sanger sequencing) and next-generation sequencing with deep sequencing for the sensitive detection of rarer mutations and cloning for an in-depth characterisation of novel viral strains of interest. NGS method was performed at the PHE Colindale laboratory in London.

2.7.3 Specimen requirements, means of identification and collection procedure:

EDTA plasma samples were extracted as soon as possible or aliquoted into sterile 2ml ampoules stored at -70 ^{o}C, as previously discussed.

Universal precautions were applied to all specimens. Personal protective equipment is worn when handling samples and Class 1 biosafety cabinet used where appropriate.

2.7.4. Instrumentation, equipment and special supplies:

• Pipettes	• Qiasymphony	• 9700 and 2720 thermalcyclers	• 3130 genetic analyzer
• Sterile tips with filters	• PCR reaction tubes	• pack	• Geneflow gel tank and Consort electrophoresis power
• Vortex	• Plate centrifuge	• Qiacube	• GeneSeq software for gel analysis

Table (2-3) Instrumentation, equipment and special supplies for Sanger method.

2.7.5. Reagents:

New Qiagen RT-PCR and HotStart Taq master mix kits which are stored at (-20^{o}C) were used.

New primers are kept at +4^{o}C. Once re-hydrated with nuclease-free water to a stock concentration of 100µM, further dilutions are made to 10µM and aliquots of the 10µM primer mixes along with the 100µM stock solutions are stored at -20^{o}C.

2.7.6. HIV-RNA extraction (purification):

RNA was purified from aliquots (1.2 ml) of plasma using the DSP Virus Cell-Free 1000 protocol instructions on the automated QIAsymphony® extraction platform (Qiagen, Crawley, UK) according to the manufacturer's description. RNA was eluted into 110 µl volume of elution buffer.

2.7.7. PCR:

A nested PCR protocol was used to amplify HIV-1 protease and reverse transcriptase regions.

The Qiagen One-Step RT-PCR kit was used for first round amplification. 20µl of extracted RNA was added to a PCR containing 1 µl primers Pin16 and Prot3 in a final volume of 50µl. Reactions were desiderated at 95 ^{o}C for one minute and cooled to 50 ^{o}C for five minutes before addition of the reverse transcriptase enzyme. Then the following thermocycle conditions were used.

1 cycle of	**40 cycles of**	**1 cycle of**
95 ^{o}C 1 min	94 ^{o}C 30 sec	72 ^{o}C 10 min
50 ^{o}C 35 min	54 ^{o}C 30 sec	4 ^{o}C hold
95 ^{o}C 15 min	72 ^{o}C 1 min 20 sec	

2.7.8 Nested Protease and Reverse Transcriptase PCRs

2ul of primary RT-PCR product has been used as template in 2 second round PCRs using primers Pin 3 and Pin 4 to amplify protease region and primers Pin 18 and Pin 2 to amplify the HIV-1 reverse transcriptase region. Second round thermo cycle conditions were as follow:

1 cycle of	40 cycles of	1 cycle of
95 °C 15 min	94 °C 30 sec 54 °C 30 sec 72 °C 1 min	72 °C 10 min 4 °C hold

2.7.9. PCR Product Quantification :

PCR products for protease (457 bp) and reverse transcriptase (804 bp) were visualised using a 1.5% agarose gel on a gel documentation (gel doc) system and GeneSeq software Figures 2-1 and 2-2.

2.7.10. Big Dye Cycle Sequencing PCR:

Primers Pin4, Pin4B and Pin3 were used to primer protease sequencing resistance, whilst primers Pin18, Pin2, Pin9 and Pin8 for the reverse transcriptase. The primer sequences are available upon request from Liverpool Specialist Virology centre. Then 19µl of each master mix (MM) were added into a 96-well PCR reaction plate in a rack. Secondary PCR product (1µg) was added to Big Dye V1.1 (Life Technologies, Paisley, UK) sequencing reactions in a final volume of 20µl using the amplification thermaocycle conditions below:

1 cycle of	**25 cycles of**	**1 cycle of**
95 °C 1 min	96 °C 10 sec 50 °C 5 sec 60 °C 4 min	4 °C hold

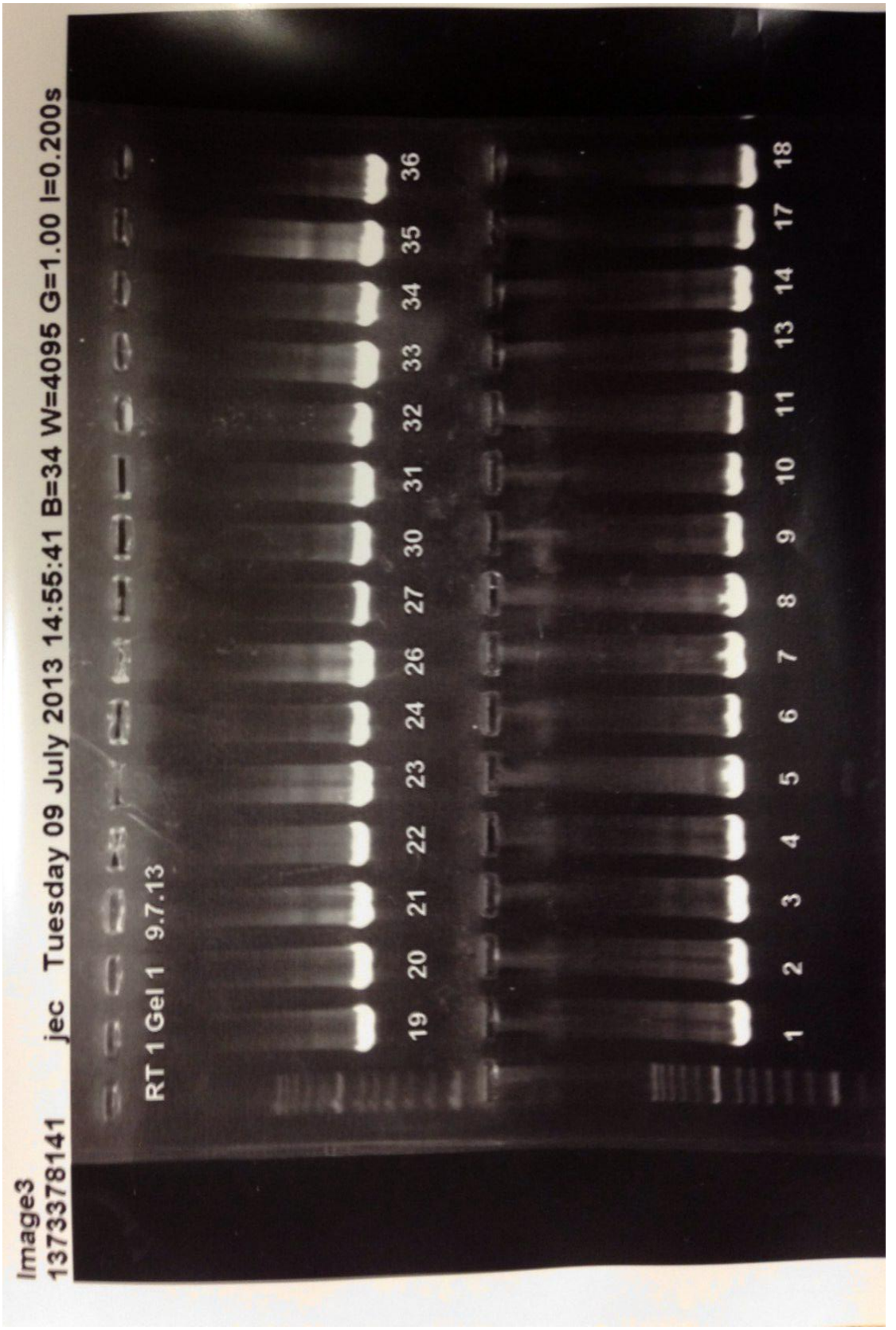


Figure (2-1) Band intensities for Reverse transcriptase (804 bp) all channels showed protein band of RT samples 1-36

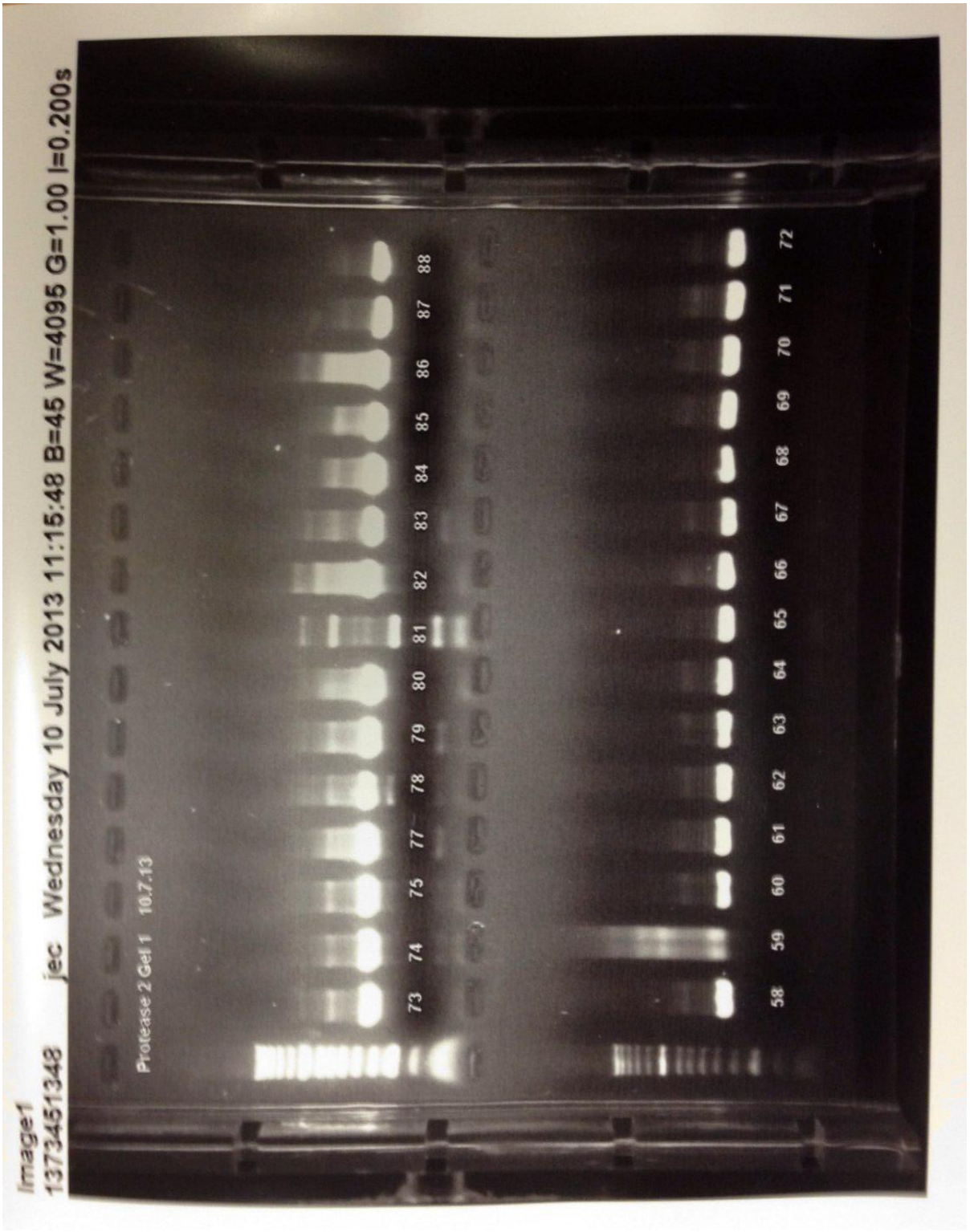


Figure (2-2) Band intensities for Protease (457 bp) all channels showed protein band of Protease samples 58-88

2.8. Data Collection Software and Data Analysis:

SA3500 data Collection software V3.0 (2009) was used for sequencing data collection and SeqScape software V2.7 (2009) was used for sequencing data analysis.

2.9. Sample size and ethics:

Currently, 1383 patients have attended the HIV clinic at King Saud Hospital, the main centre of the Saudi programme for HIV/AIDS patients in Jeddah region in the past decade. The proportion of patients currently receiving antiretroviral therapy is about 75%.

This sample size has been calculated by using the national statistical services (NSS) sample size calculator in the World Wide Web (NSS 2012).

The estimated rate of ARV resistance in ART naïve HIV patients is about 6.4 %, based on a single publication from Oman (Al Dhahry et al. 2004).

The sample size was calculated, aiming for 95% confidence and a precision of +/- 0.02. A sample size of 109 treatment naïve HIV positive patients is thus expected to include about 8 ARV resistant and 101 non-ARV resistant patients. These numbers will allow the true ARV resistance prevalence rate to be estimated with an exact binomial 95% confidence interval of 0.09 (0.46 to 0.59).

The study was approved by the Research and Ethics Committee of the Liverpool School of Tropical Medicine (reference 12-15, Appendix E) and

the Institutional Review Board of King Fahd Medical City, Riyadh for the Ministry of Health (reference 12-159, Appendix E).

2.10. Next Generation Sequencing methodology:

Samples were processed for deep sequencing by Next Generation Sequencing (NGS) at the Public Health England Laboratory in Colindale London, UK. Samples were processed according to the following protocol:

2.10.1 RNA extraction

200 μl of each sample was extracted using QIAamp UltraSens Virus Kit (QIAGEN) as per the kit instructions and eluted into 60μl.

2.10.2 PCR amplification

1.3kb region of the HIV *pol* gene was amplified by single-step reverse transcription (RT) first round PCR followed by a nested PCR. RT-first round PCR was carried out using One-Step RT PCR kit (QIAGEN) using 10μl of extract in 50μl reactions containing 0.3μM of each primer, according to the manufacturer's instructions (forward primer, P1: TGA ARG AIT GYA CTG ARA GRC AGG CTA AT; reverse primer, R2: CCT CIT TYT TGC ATA YTT YCC TGT T).

The cycling conditions were 50◦C for 40 minutes, 95◦C for 15 minutes followed by 35 cycles of 94◦C for 30 seconds, 53◦C for 30 seconds, 72◦C

for 1 minute and then 4 minutes at 72◦C. One µl of first round products were carried forward into 50µl nested PCR reactions containing 0.3µM of each primer using Platinum Taq (Life Technologies).

Nesting primers were P7: CTT TAR CTT CCC TCA GAT CAC TCT, R8: GGC TCT TGA TAA ATT TGA TAT GTC CAT. The cycling conditions used were 95◦C for 5 minutes then 35 cycles of 90◦C for 30 seconds, 50.3◦C for 30 seconds, 72◦C for 1 minute followed by a further 2 minutes at 72◦C.

2.10.3 Library preparation for sequencing

PCR products were visualised using agarose gel electrophoresis and then purified using QIAQuick kit (QIAGEN). DNA was quantified using both Qubit® dsDNA Broad Range and High Sensitivity Assay Kits and the Qubit® 2.0 Fluorometer (Life Technologies). One ng/µl of the amplified DNA was used for library preparation with the Nextera XT DNA sample prep kit (Illumina) as per the kit protocol. Next generation sequencing was performed on the MiSeq system (Illumina).

2.10.4 Bioinformatic analysis.

Following the generation of the paired end reads, a subset of the reads from each fastq file was compared to a local database of HIV reference sequences using BLAST in order to identify an optimum reference sequence in preparation for mapping. Reference mapping was then performed using BWA-MEM version 0.7.5. Utilising SAMTools the resulting files were then converted into BAM format in preparation for in-house developed software, QuasiBAM which generates consensus sequences of the protease and reverse transcriptase regions and produces detailed information on the frequencies of minority variants present within each sample. These procedures were all automated using a computational pipeline developed in-house using Python and C++.

2.11. Definitions:

2.11.1. Primary HIV drug resistance:

Drug resistant HIV that results from becoming infected with a virus that is already resistant to one or more drugs (Geretti & Easterbrook 2001)

2.11.2. Treatment emerging HIV Drug Resistance

Drug resistant HIV that develops during ART treatment

2.11.3. Recruitment and tests

During the 3 month study period (November 2013 – February 2014), 664 different Saudi patients attended the clinic and 116 were identified as having never received ART. Of these 116, 109 (94%) agreed to take part in the study. The remaining 7 were not processed because 4 patients refused to join the study and 3 samples had a low viral load and we could not process the genotypic resistance test. Paired data and blood samples were obtained from these 109 and ultimately genotypic resistance testing was successful in 96 (88%) of patients and results were obtained by next generation sequencing in 105 (96%) patients. Resistance tests were only completely unobtainable from 4 (3.7%) patients (Figure 2-3).

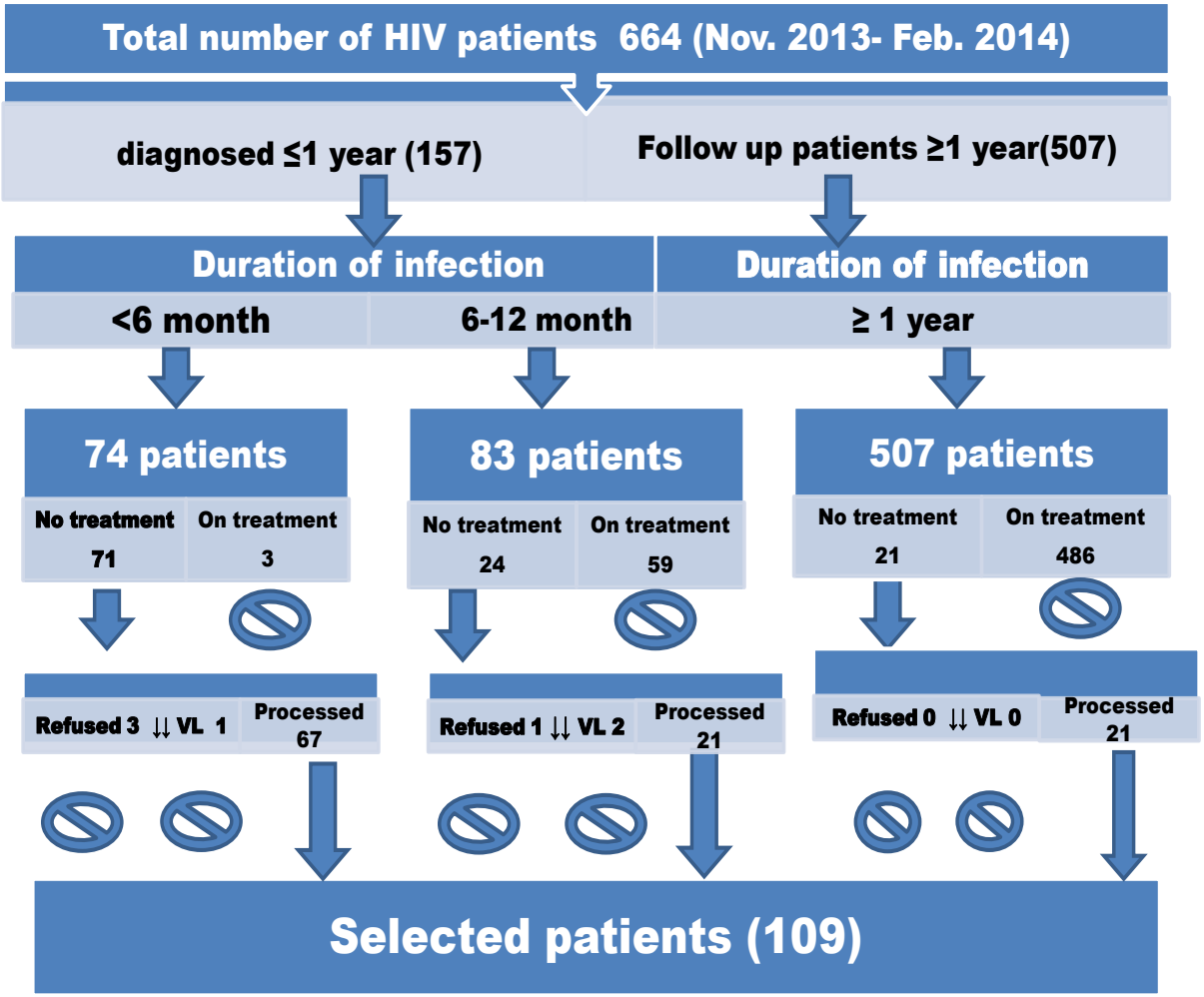


Figure (2-3) Patient selection for ART resistance testing (VL= insufficient viral load for testing)

Chapter 3

Clinical features and epidemiology of HIV in Jeddah

3.1. Introduction

As discussed in chapter 1, there are few systematic descriptions of HIV in Arabic countries including the Kingdom of Saudi Arabia, although a preliminary clinical report was published in 1993 (Ellis et al) and in 2004 (Madani, Al-Mazrou et al. 2004) and more recently there have been publications on updated epidemiology in the Kingdom (Kabbash, Felemban et al. 2012). There had been no studies on prevalence of coinfection with TB or hepatitis and risk factors for such condition and no assessment of ART use.

The aims of this study were to:

1. Describe the demographic and clinical profile and laboratory features of a large cohort of HIV positive Saudi adults.
2. Describe the prevalence of coinfections and the risk factors in the same cohort.
3. Describe the use of ART and resistance tests in the cohort.

The setting for the study is the largest HIV clinic in the Kingdom, at King Saud Hospital, Jeddah.

3.2. Results:

Data were collected for all 1383 HIV/AIDS patients who were followed at King Saud Hospital under the Saudi Ministry of Health HIV/AIDS programme in Jeddah, between 2000 and 2010. Three quarters 1026/1383 (74.2%) of the patients were males.

3.2.1. Age and features of HIV infection

The youngest patient was 18 years old and the oldest was 86 years, while the oldest age at diagnosis of HIV infection was 80. The mean (SD) age of patients was 41 (11.9) years and the median (range) was 40 (18-86) years. The mean (SD) age at HIV/AIDS diagnosis was 36.7 (11.4) years and the median (range) 35 (18-80) years.

The patients were divided into five main categories according their age group. The first age category contains patients aged 18-25 years, the second 26-35 years, the third age category aged 36-45 years, patients aged 46-59 years and finally patients aged 60 years or older. Most of the patients (64%) fall in the middle two categories i.e 25-45 years old. Only 0.1% out of the total patients were older than 60 years. About 9% of the patients were young (18-25 years) males.

HIV infection duration was categorised into 4 groups which included a recent infection group 1-2 years, 2-5 years, 6-10 years and lastly patients who had HIV for more than 10 years.

Most of the patients included in this study were within the second and third groups. This means that most of the patients (93%) had been infected with HIV for 2-10 years. Only 1% of patients had HIV infection for more than 10 years and 6% were recently diagnosed (Table 3-1).

HIV duration of infection		Female		Male		Total	
		n/N	%	N/n	%	n/N	%
	1-2 years	26/357	7.3%	60/1026	5.8%	86/1383	6.2%
	2-5 years	159/357	44.5%	386/1026	37.6%	545/1383	39.4%
	6-10 years	168/357	47.1%	570/1026	55.6%	738/1383	53.4%
	10+ years	4/357	1.1%	10/1026	1%	14/1383	1%
	Total	357	100%	1026	100%	1383	100%
Total		357		1026		1383	100%

Table (3-1) HIV infection duration (n=1383)

3.2.2. Educational level:

Data on educational level were obtained for most of the patients but was not available for about 20% (286/1383).

Poor education level was relatively common among these patients. More than quarters of the patients were illiterate, 27.4% (300/1097). A smaller proportion can read and write but have not achieved high school level. This was almost equally distributed between the two genders. More than half of the patients, 56% (620/1097), had at least a high school certificate. University degree holder or equivalent

composed about 5% (55/1097) of all HIV/AIDS patients with no significant gender difference (Table 3-4).

3.2.3. Marital status:

Over a third of the patients 488/1383 (35.3%) were single and most of these were men 435/488 (89%). The difference between single males and females was highly significant (P<0.001, OR 0.24, 95% CI 0.17-0.33). 52.6% (727/1383) of the patients were married with significantly more married women (65.3%) than men (48.1%). 6% of all patients were divorced with no significant gender difference. More women (13.2%) have been widowed than men (3%) (Table 3-4)

3.2.4. Reasons for HIV testing:

The reasons for testing were not mutually exclusive, so results were reported and differences were analysed individually (Table 3-3).

More than 57% (795/1483) were tested after being clinically suspected to have HIV infection after a visit to a medical centre. There was no significant difference between the two genders, in males 56% (578/1026) and in females 60% (217/357) (P= 0.14, OR 1.2, 95% CI 0.93-1.5).

3% (47/1383) of the patients were discovered to have HIV infection through premarital testing. 4% (37/1026) of males had a positive HIV

premarital test, twice as many as females 9/357 (2%), (p=0.3, OR 0.69, 95% CI 0.33-1.44), although numbers are too small to reach significance (Table 3-3).

More than half of the patients 51% (705/1383) were found to be HIV positive as part of routine blood tests, with equal proportions of males (52%) and females (46%), (P= 0.06, OR 0.8, 95% CI 0.62-1.02).

8% (110/1383) of patients were found to be HIV positive after they had preoccupation medical tests, with no gender difference.

3.2.5 Risk Factors for HIV:

Probable heterosexual transmission was the most likely route in over half of the patients (709/1383). (Table 3-3, Figs 3-1A to 3-1C).

Homosexual transmission, male to male (MSM) was the main route in 26% of men (264/1026).

23% (301/1288) were known to have unprotected sex with an HIV positive partner. 44% (133/301) of patients with a history of not using protection during sexual activity were men and women accounted for 66%, although only 24% of the whole cohort were females (Table 3-2). Most of the females admitted to direct sexual contact with HIV positive patients. This was reported in the past medical history of 70% (210/301) of the women.

Around 7% (97/1329) of patients had a history of recent (in past 5 years from the time of data collection) use of injecting drug (IDU) and most of these were males ie 81% (79/97).

10% (148/1383) had a previous history of blood transfusion. About 13% (46/357) of the females had received blood transfusion in comparison to 10% (102/1026) of the male HIV/AIDS patients, with no significant gender difference (Table 3-2).

About 7% (98/1383) of the patients had previously (in past 5 years from the time of data collection) been in prison. Again, those were predominantly males 73/98 (75%).

A past medical history of having sexually transmitted infections (STI) was recorded in 6.4% (88/1383) of patients, with similar gender distribution, in about 6.5% (67/1026) of the males and 5.9% (21/357) in females.

A past medical history of mental illness was found in 4% (55/1383) of all patients. A history of overseas travel was recorded in about 13% (179/1383) of patients. The proportion was similar in males and female (Table 3-2).

	Sex										
	Female		Male		Total						
	n / N	%	n / N	%	n / N	%	No data (Missing)	X^2	P	OR	95%CI
Heterosexual	71/357	19.9%	638/1026	62.2%	709/1383	51.3%	0	189.63	0.00	0.1	0.1-0.2
MSM	0	0%	264/1026	26%	264/1026	26%	0	-	-	-	-
IDU	18/330	5%	79/999	7.9%	97/1329	7%	54/1383 (4%)	2.2	0.13	0.6	0.3-1.1
Blood products	46/357	12.9%	102/1026	10%	148/1383	10.7%	0	2.4	0.12	1.3	0.9-1.9
Unprotected sex	168/310	54%	133/978	13%	301/1288	23%	95/1383 (7%)	216.6	0.00	7.5	5.6-10
Contact with HIV	210/361	58.8%	91/997	9%	301/1358	22%	25/1383 (2%)	369.5	0.00	13.8	10.2-18
History of prison	25/357	7%	73/1026	7.1%	98/1383	7.1%	0	0.01	0.94	0.9	0.6-1.6
History of STI	21/317	6.6%	67/985	6.8%	88/1302	6.7%	81/1383 (6%)	0.01	0.91	0.97	0.5-1.6
Overseas travel	39/357	10.9%	140/1026	13.6%	179/1383	12.9%	0	1.74	0.18	0.7	0.5-1.1

Table (3-2) HIV Risk factors in Jeddah, Saudi Arabia. Data on risk factors are not mutually exclusive, so totals are more than 100%

		n/N	%
Education level	Illiterate	300/1097	27.4%
	Read and write	122/1097	11.1%
	High school	620/1097	56.5%
	University	55/1097	5%
	PG	0	0
	Missing Data	286/1383	20.7%
Marital status	Single	488/1383	35.3%
	Married	727/1383	52.6%
	Divorce	89/1383	6.4%
	Widow	79/1383	5.7 %
Reasons for HIV testing	Clinical suspicion	795/1383	57.5%
	Routine serology	705/1383	51%
	Premarital test	46/1383	3%
	Preoccupation	110/1383	8%
Presenting history	Respiratory	611/1383	44.2%
	Asymptomatic	408/1383	29.3%
	GI	312/1383	22.6%
	Skin & MS	45/1383	3.3%
	CNS	4/1383	0.3%
	Genitourinary	4/1383	0.3%
	CVS	2/1383	0.1%
Risk factors	Heterosexual	709/1383	51.3%
	MSM	264/1026	26%
	IDU	97/1383	7%
	Blood Transfusion	148/1383	10.7%
	Unprotected sex	301/1383	21.8%
	Contact with HIV	301/1383	21.8%
	History of prison	98/1383	7.1%
	STI	88/1383	6.4%
	Overseas travelling	179/1383	12.9%
HIV duration of infection	1-2 years	86/1383	6.2%
	2-5 years	545/1383	39.4%
	6-10 years	738/1383	53.4%
	10+ years	14/1383	1%
	Total	1383	100%

Table (3-3) Demographic and clinical features results summary

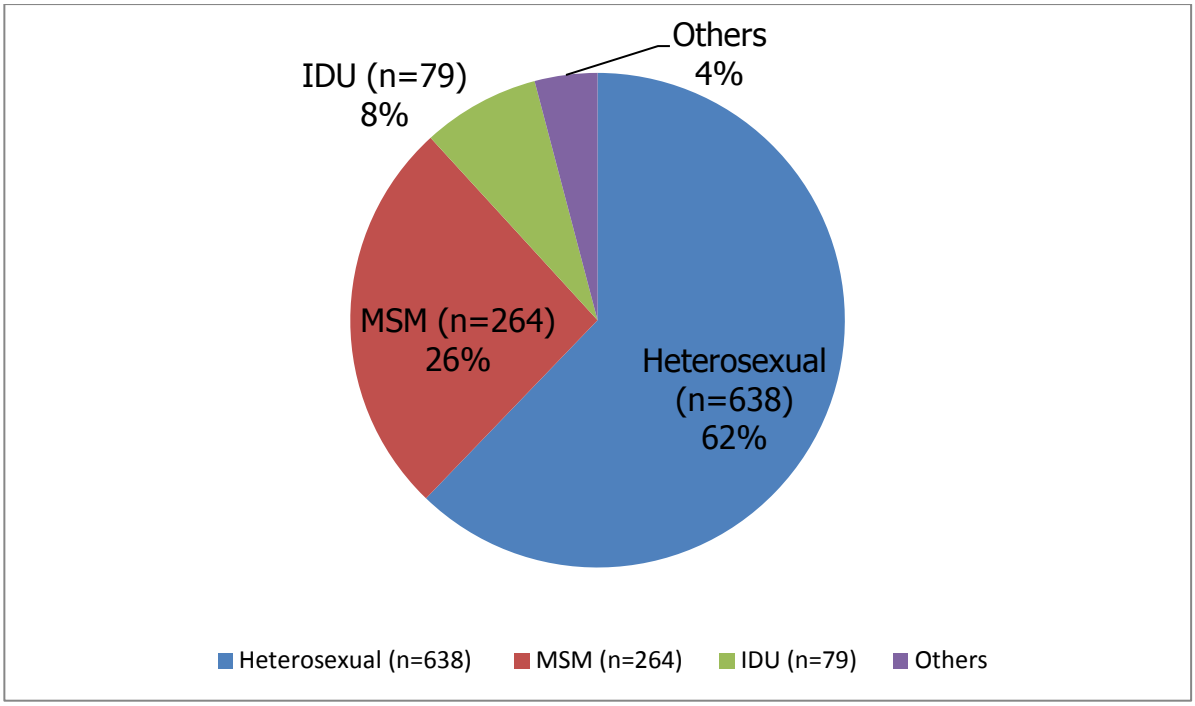


Figure (3-1A) HIV main risk factors (males, n=1026)

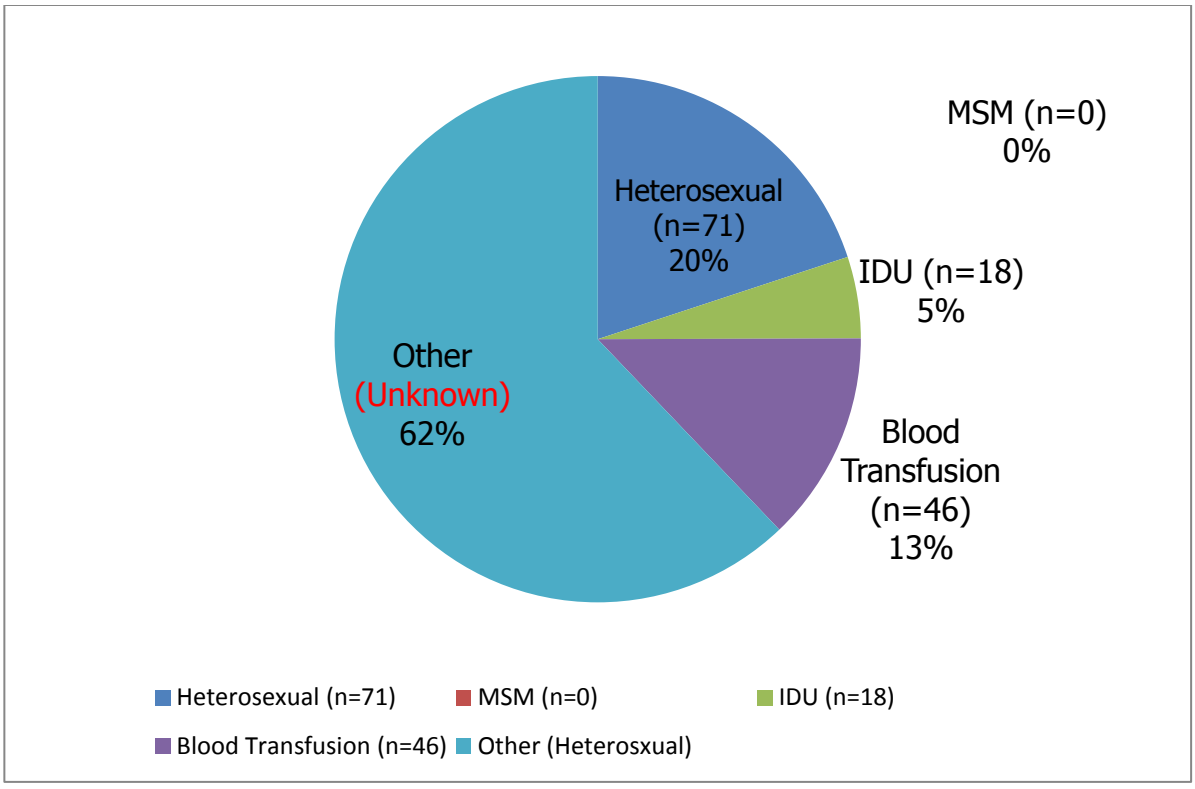


Figure (3-1B) HIV main risk factors (females, n=357)

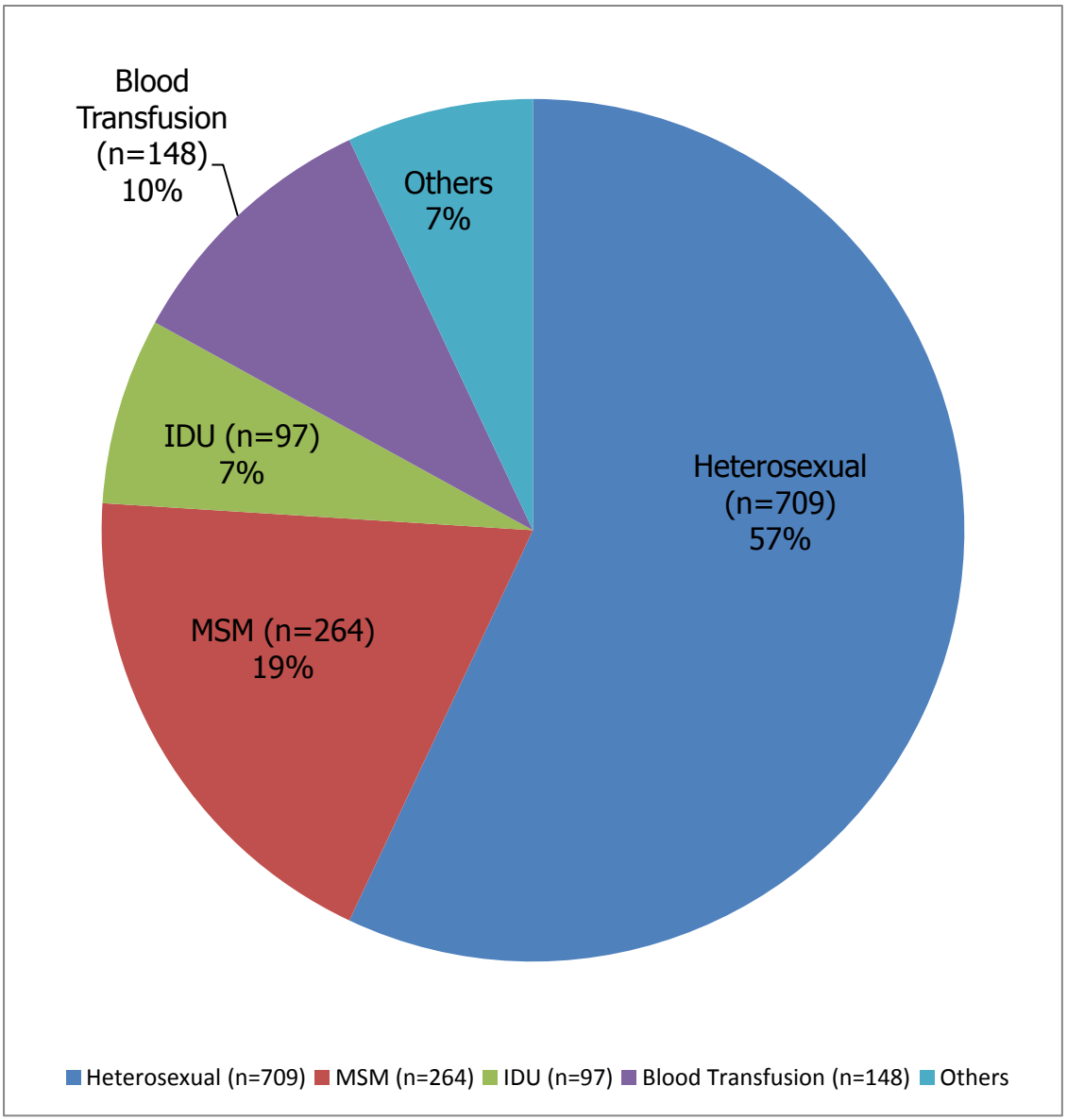


Figure (3-1C) HIV main risk factors (whole cohort, n=1383)

3.2.6. Presenting history:

Patients were divided in this study according to their main presenting symptoms at the time of diagnosis table (3-4). The most common was respiratory symptoms (44%). 29% (408/1383) of patients were asymptomatic. Patients who presented with gastrointestinal (GI) symptoms made up the third most common presenting group (22%). All other presenting symptoms including central nervous system (CNS), skin and musculoskeletal system, cardiovascular system (CVS), and genitourinary system (GUS) were present in a minority of the patients with total percentage less than 4% (56/1383).

		Sex									
		Female		Male		Total					
		n/N	%	N/n	%	n/N	%	X^2 *	P *	OR	95%CI
Education	Illiterate	61/287	21.3%	239/810	29.5%	300/1097	27.4%	7.2	0.007	0.64	0.4-0.88
	R&W	37/287	12.9%	85/810	10.5%	122/1097	11.1%	1.3	0.3	1.26	0.83-1.9
	high school	174/287	60.8%	446/810	55%	620/1097	56.5%	2.67	0.1	1.27	0.9-1.6
	University	15/287	5.2%	40/810	4.9%	55/1097	5%	0.04	0.84	1.06	0.55-2.02
	PG	0	0	0	0	0	0	-	-	-	-
	Missing Data	70/357	19.6%	216/1026	21%	286/1383	20.7%	-	-	-	-
Reasons for HIV testing	Clinical suspicion	217/357	60.8%	578/1026	56.3%	795/1383	57.5%	2.14	0.14	1.2	0.93-1.55
	Routine serology	167/357	46.8%	538/1026	52.4%	705/1383	51%	3.39	0.065	0.8	0.62-1.02
	Pre-marital test	9/357	2%	37/1026	4%	46/1383	3%	0.97	0.3	0.69	0.33-1.44
	Preoccupation	25/357	7%	85/1026	8%	110/1383	8%	8.2	0.004	0.64	0.46-0.88
Marital status	Single	53/357	14.8%	435/1026	42.4%	488/1383	35.3%	88.04	0.00	0.24	0.17-0.33
	Married	233/357	65.3%	494/1026	48.1%	727/1383	52.6%	31.12	0.00	2.02	1.5-2.6
	Divorce	24/357	6.7%	65/1026	6.3%	89/1383	6.4%	0.07	0.79	1.07	0.6-1.7
	Widow	47/357	13.2%	32/1026	3.1%	79/1383	5.7 %	49.6	0.00	4.7	2.8-7.7
presenting history at diagnosis	asymptomatic	118/357	33.1%	287/1026	28%	408/1383	29.3%	-	-	-	-
	GI	81/357	22.6%	231/1026	22.5%	312 /1383	22.6%	-	-	-	-
	Resp.	141/357	39.5%	470/1026	45.8%	611/1383	44.2%	-	-	-	-
	CNS	0	0	4/1026	0.4%	4/1383	0.3%	-	-	-	-
	CVS	1/357	0.3%	1/1026	0.1%	2/1383	0.1%	-	-	-	-
	Skin & MS	14/357	3.9%	31/1026	3%	45/1383	3.3%	-	-	-	-
	Genitourinary	2/357	0.6%	2/1026	0.2%	4/1383	0.3%	-	-	-	-

Table (3-4) HIV features in Jeddah, Saudi Arabia (n=1383)

*(Uncorrected X^2 and two tailed P)

Toxoplasma serology (IgG) serology was positive in 45% of the tested patients (311/684) but was missing in more than half of the patients. The difference between males (238/495, 48%) and females (73/189, 38.66%) was significant (P= 0.03, OR 0.68, 95% CI 0.48-0.97). Cytomegalovirus (CMV) infection was tested in 65% (897/1383) of the patients and 70% (634/897) were positive with no significant difference between genders. Only 44.5% (616/1383) of patients were tested for Varicella Zoster Virus (VZV) antibodies. 33.4% (205/616) were positive with no difference between males at 34.6% (158/457) and females at 29.6% (47/159) (P =0.24, OR 0.79, 95% CI 0.53-1.2). Epstein - Barr virus (EBV) infection was tested in about 57% (788/1383) patients and was positive in 67% (531/788).

60% (830/1383) of patients were tested for syphilis using the Venereal Disease Research Laboratory (VDRL) test and 15% (128/830) were positive. This included 16.5% (100/605) of males and 12.4% (28/225) of females (P>0.14, OR 0.72, 95% CI 0.45-1.15). Missing data and summary data are detailed in (Table 3-5).

Although the proportion of males and females with positive VDRL were similar, there was significantly higher VDRL positivity rate in MSM (24.2%, 41/170) compared to other men (15%, 129/856) (X^2=8.3, P=0.003, OR 1.79, CI 95% 1.2-2.6)

	Sex							
	Female	Male	Total	Missing Data				
	n/N (%)	n/N (%)	n/N (%)	n/N (%)	X^2	P	OR	95%CI
Toxo.	73/189 (38.6%)	238/495 (48%)	311/684 (45.5%)	699/1383 (50.5%)	4.93	0.03	0.68	0.48-0.97
CMV	159/242 (65.7%)	475/655 (72.5%)	634/897 (70.7%)	486/1383 (35%)	3.96	0.05	0.73	0.52-1.01
EBV	142/220 (64.5%)	389/586 (66.4%)	531/788 (67.4%)	595/1383 (43%)	1.12	0.28	0.84	0.6-1.18
VZV	47/159 (29.6%)	158/457 (34.6%)	205/616 (33.3%)	767/1383 (55.5%)	1.34	0.24	0.79	0.53-1.2
VDRL	28/225 (12.4%)	100/605 (16.5%)	128/830 (15.4%)	553/1383 (40%)	2.1	0.14	0.72	0.45-1.15

Table (3-5) past and recent infections in HIV patients (denominators vary for each test) Toxo= Toxoplasmosis

3.2.7. Laboratory results:

The mean haemoglobin (Hb) was 12.9 mg/dl. The mean (SD) count lymphocyte count was 41(8)% and the median count 43%.The mean (SD) absolute $CD4^+$ cell count was 438 (339) cells/mm^3 and the median was 374 cells/mm^3, and the mean (SD) absolute $CD8^+$ cell count was 1166(699) cells/mm^3. The median was 1052 cells/mm^3.

	Mean	SD
Age	40	11.8
Age at diagnosis	35	11.4
Duration of HIV infection	6	2.6
Baseline Hb	13	12.1
Baseline CD4+	374	339
Baseline Lymphocyte %	43	8.2
Baseline Viral Load (log)	3.3	1.5

Table (3-6) Haemoglobin and virology results at presentation of all HIV patients (n=1383)

Table (3-7) summarise the CD4+ cell count and HIV viral load results in HIV patients in relation to presenting symptoms, showing lower CD4+ counts and high viral load in those with cardiovascular presentation and lower CD4+ counts with CNS disease and GU symptoms (all small numbers of cases). The relationships of CD4+counts and viral load to coinfections is presented in Chapter 4.

		Absolute CD4+		VL(log)	
		Mean	SD	Mean	SD
Sex	Female	471	316	3.18	1.47
	Male	427	346	3.41	1.52
presenting Hx	Respiratory	396	276	3.33	1.53
	GI	431	416	3.45	1.53
	Skin&MS	428	306	3.18	1.50
	CNS	270	77	3.61	1.25
	CVS	149	97	4.67	0.039
	Asymptomatic	513	354	3.29	1.46
	GU	240	229	3.11	1.62

Table (3-7) CD4+ cell count and HIV viral load (VL) in relation to presenting symptoms

3.2.8. Viral load:

The mean (SD) log HIV viral load was 3.35 (1.5) copies/ml. The minimum observed log viral load was 1.3 and the maximum was log 6.8 copies/ml.

3.2.9. IDU and History of prison:

12% (12/97) of IDU patients also had history of being in prison, the results showed a significance different in IDU imprisonments to non-IDU patients (P= 0.03, OR 1.9, 95% CI 1.03-3.74).

			History of prison		Total
			Yes	No	
IDU	Yes	Count	12/97(12.4%)	85/97(87.6%)	97
	No	Count	86/1286 (6.7%)	1200/1286 (93.3%)	1286

Table (3-8) IDU and history of prison (n=1383)

3.3. IDU and STI:

About 10% STI patient had a history of IDU, of 97 STI patients 9 patients were injecting drug users (9/97, 9.3%)

3.4. Patients taking ART:

Overall, 75% of patients had received ART (1049/1383).

3.5. WHO classification:

Most of the patients (60%) presented to the clinic in WHO stage (1). The second common presenting stage was (2) with 32%. Only 8.5% (118/1383) were in stage (3) and 0.4% (5/1383) in stage 4 at presentation.

Almost half of the females were presented to HIV clinics when they were in stage 1 and 40% (143/357) presented while they in stage 2. Only 8.9% (34/357) were in stage 3 or 4 at the time of presentation.

Most of the males presented to HIV clinics while they are in stage 1 (62%), then stage 2 with about 30% (298/1026) of male patients. About 8.6% were in stage 3 or 4

			Sex		Total
			female	Male	
WHO staging at presentation	1	n/N (%)	180/357 (50.4%)	639/1026 (62.3%)	819/1383 (59.2%)
	2	n/N (%)	143/357 (40.1%)	298/1026 (29%)	441/1383 (31.9%)
	3	n/N (%)	32/357 (8.9%)	86/1026 (8.4%)	11/1383 (8.6%)
	4	n/N (%)	2/357 (0.6%)	3/1026 (0.3%)	5/1383 (0.4%)

Table (3-9) WHO staging at presentation

3.6. Discussion

3.6.1 HIV/AIDS in the Kingdom of Saudi Arabia:

Because of many religious and social concerns in the Kingdom, HIV had been difficult to tackle or even to discuss. This study is the first to look comprehensively at both epidemiology and clinical features in a cohort of HIV patients in the Kingdom of Saudi Arabia. The first part of this study was carried out retrospectively using case notes and patient details from their files, which varied in accuracy from case to case depending on case note quality. This was a significant limitation during the data collection period (secondary data limitations), although, I attempted to overcome this by reviewing all available medical notes to pick up missing or deficient data for each case.

The median (range) age for the cohort was 41 (18 – 80) years. Predominance of male gender was expected and matches previous studies in the Region and in the Kingdom. One possible explanation is that Saudi males are more exposed to risk factors including travel and they have less social pressure to seek for medical advice and treatment. Similar to this study, about two thirds of all HIV patients in a review in Saudi Arabia from 2000 to 2009 were males, with a male to female ratio of 4.4:1 (Kabbash et al. 2012).

Illiteracy was noted in about quarter of all patients, which emphasises the education challenges to implementation of HIV/AIDS prevention strategies, including safe sex education. A recent study published by Al-Othman et al. (2010) confirmed that new HIV case notification is increasing every year in the Kingdom. They suggested that the lack of detailed education about safe sex was contributing to this annual increase (Al-Othman et al. 2010).

Improving education should help to decrease HIV transmission, even in such a conservative society. Educational programmes and strategies should focus on young adults, especially males, as they represent the majority of Saudi HIV positive patients. Even though extramarital sexual relations are strictly prohibited in Saudi law and regulation, more than half of the HIV positive patients in Jeddah are single. Again, this emphasizes the reality of individual behaviours despite standard expectation of societies, and shows the need to educate single men and women despite cultural norms.

This study is useful because data on the probable route of acquiring HIV are much more complete than national studies using central notification data. Only 11% of patients had no identified route, compared to 41% in 2004 and 46% in 2012 reports (Madani et al 2004; Kabbash et al. 2012)

As expected as it is one of the most common modes of HIV transmission globally, this study showed that heterosexual transmission of HIV was noted in about half (709/1383) of the Saudi patients in the Jeddah HIV clinic, compared to about 40% in 2004 (Madani 2004). As expected, it was the main route of transmission in women, statistically more common than in men. In men, male sex with men accounted for 26% of all cases, much higher than previous estimates but similar to recent data from some neighbouring countries like Israel (Zohar et al. 2014). This is a problem as homosexual activity is not legal and it is difficult to provide education and support for this group in Arabic societies (Shawky, Soliman et al 2009; Mumtaz et al. 2011).

Travelling abroad is also an important concern in HIV positive patients and was found in 179 (13%) patients. In Saudi Arabia and by Islamic law, sexual practice is prohibited except through marriage. No sex workers are allowed in the Kingdom by law and this is strictly controlled by the Saudi authorities. Under such circumstances, travelling abroad provides an important opportunity to acquire and import HIV infection into the country. It is important to emphasise the use of protection (condoms) as about 301(22%) of patients admit practicing sexual activities with their HIV positive partner without using protection.

In Saudi Arabia blood and blood products are strictly checked for infectious disease including HIV. Cases of HIV infection transmitted via blood or blood products have hardly occurred since 2001 and all remaining cases are from previous blood or blood products transfusion before 1986. Patients who had acquired HIV from transfusion of blood products account for about 25% of cases in Madani's study (Madani 2004). In this study about 10% of patients had a history of blood product transfusion at least once in their past medical history. This is probably because most of the cohort acquired HIV later than 1990 by which time the blood transfusions were recognized and prevented in the Kingdom. National data collected from the period 2012-2013 showed that blood or blood products related HIV transmission had declined to 0.019% in Saudi Arabia (UNAIDS 2014). This decline is expected and expected to decrease more in future due to strict blood bank control.

This study showed a larger proportion of other high risk groups than previous studies in KSA.

However, a past medical history of STI was rarely reported in HIV positive Saudi patients, being found in only 88 (6.4%) of patients. This suggest a large amount of underdiagnoses, as many studies from the West and from other Regions such as Africa show rates of STI infections of about 15% and 50% respectively (Hegazi et al. 2015; Winston et al. 2015)

In this cohort only 60% had records of any syphilis serology being performed and there was no specific protocol to encourage routine examination and testing for STI in the clinic. As STI are important coinfections in transmission of HIV to others, this aspect of HIV care needs more prominence in the national HIV programme, despite the cultural difficulties of such issues.

The VDRL test was positive in 128 (15%) but there was no statistically significant difference between males and females, except in men who have sex with men MSM (24.2%, 41/170) compared to other men (15%, 129/856) (X^2=8.3, P=0.003, OR 1.79, CI 95% 1.2-2.6) as expected from many studies elsewhere (Maek-a-nantawat 2014). The tests used in clinics were very basic and the difficulties of interpretation test like VDRL in a country where endemic syphilis is also present have already being discussed (Marks, Solomon & Mabey 2014). This study suggests that, along with improving protocols for testing HIV positive patients regularly for STI's as in other countries (BHIVA 2013), the methodologies for

screening for syphilis should be reviewed to include more modern serological test such as *Treponema pallidum* particle agglutination assay (TPPA) or *Treponema pallidum* hemagglutination assay (TPHA) and more vigorously implemented. This is particularly important in MSM and other patients for whom risky sexual practices and more frequent partner change may be a feature.

A past history of IDU was found in 97 patients (7%), compared to 1.3% a decade ago (Madani 2004). As expected in a conservative community, IDU in HIV positive patients was significantly associated with male gender. It is a concern that HIV is being spread this way as Jeddah is known to have a substantial larger population of IDU than elsewhere in the Kingdom, but in whom previous studies had shown very little HIV infection. However, more recent studies confirm that this is changing in Saudi Arabia with 10% of heroin users in Riyadh recently reported to be HIV positive (Al-Shomrani 2014). In Iran HIV is well known to be associated with IDU as an important risk group, and this is difficult to access for education as IDU activity is illegal. If rates continue to raise this could become a major public health problem in KSA.

98 (7%) of HIV/AIDS Saudi patients had a history of being in prison. This is may be a chance association but probably suggests that being in prison should be considered as an important risk for HIV. It may be an independent risk factor for HIV than IDU, as only (12/98, 12.2%) IDU had been in prison. This ratio is less than might be expected, as in

many countries up to 50% of IDU have a history of being in prison (Hope et al. 2013).

One of the important national programmes in Saudi Arabia is the premarital testing programme for certain inherited and infectious diseases. This programme is compulsory for all Saudi couples who are intending to get married. In this cohort about 3% of cases had been diagnosed to have HIV infection through this programme. This screening programme should be enhanced to lead to early detection of cases, particularly in antenatal care where HIV screening is not yet routinely applied despite the benefits in reducing mother to child transmission (Ross et al. 2015).

57% of patients were clinically suspected to have HIV infection after a visit to a medical centre, which reflects the importance of keeping all medical centres and their personnel alert to maintain this high quality level of practice. Most of the patients included in this study presented to the clinics with respiratory symptoms (44%) and gastrointestinal (GI) symptoms (22%), while about 30% were asymptomatic. In 2004 Madani et al found that asymptomatic presentations were more common and represented about 70% of HIV positive patients in the whole Kingdom (Madani 2004). This may be explained by the improvement in patient's awareness of HIV symptoms and supports the presence of well-developed HIV/AIDS national programmes; so that

patients may they seek medical advice immediately for minimal symptoms rather than ignoring these and presenting later.

All other presenting syndromes including central nervous system (CNS), skin and musculoskeletal system, cardiovascular system (CVS), and genitourinary system (GU) symptoms were presenting feature in only a small minority of patients, accounting for less than 4% (56/1383) of the whole cohort.

The HIV clinic in Jeddah plays an important role in supporting HIV positive individuals and improving their health as the median of HIV infection duration was about 6 years and the highest duration of HIV infection was 18 years among patients who were being following at this clinic.

After this study was performed in 2011, another HIV cohort review was performed at King Faisal Specialist Hospital in Riyadh, including all patients following in their clinics or admitted to the hospital between 1984 and 2012 (Al-Mozaini et al. 2014). Some features of that smaller cohort are compared to those in Jeddah cohort in Table 3-10.

There are interesting differences as the King Faisal Hospital cohort is in a different city and is a tertiary referral centre for the Kingdom, while our study looked at the whole population attending a local major centre in Jeddah. For example, I found more IDU in Jeddah than in Riyadh as expected from the known larger number of IDU in Jeddah (Berger 2015). However, HCV coinfection was more common in Riyadh,

probably because it included early patients infected by blood products in the 1980's and despite there being more IDU in Jeddah, a known risk factor for HCV/HIV coinfection (Berger 2015).

Character	Riyadh study	This study	Remarks
Place	Riyadh	Jeddah	Most of HIV patient are in Jeddah
Hospital	King Faisal Hospital	King Saud Hospital	KFSH is a tertiary referral hospital for whole of KSA. KSH is MOH and open to public
Patients	602 patients	1383 patients	
Period	1984-2012	2000-2010	Jeddah more recent cohort overall
Male/females	69% male	74% male	Similar results
Mean age (SD) at HIV diagnosis	30 (14) years	36 (11) years	Similar results
Married	48%	52%	Similar results
Heterosexual	54%	57%	Similar results
Bisexual & MSM	3.3% +1.66%	MSM 26%	More MSM in Jeddah
IDU	2.82%	7%	More IDU in Jeddah
Blood transfusion	14%	10.7%	Similar results
HCV coinfection	12.9%	6%	More HCV in Riyadh
Treated (HAART)	71%	75%	Similar results

Table (3-10) Comparison with a recently published report in cohort of HIV positive patients in Riyadh (Al-Mozaini et al. 2014)

Chapter 4

HIV coinfections

4.1 Introduction

As elsewhere in the Region, Saudi Arabia has obvious gaps in epidemiological data about HIV and even less information about clinical features related to HIV infection. Coinfections with TB, HCV and/or HBV are particularly important for HIV patients for different reasons, but very few data are available about these in KSA.

Moreover, data about risk factors and assessment of the actual situation for high risk and hard to reach groups of patients are poor and need to be improved.

This section focuses on the prevalence of key coinfections in the HIV cohort in Jeddah, and the behavioural risk factors associated with them. The aim was to provide data on efficiency of the service for detecting coinfection, to provide an estimate of patients who might need further specific treatment and recommendations on whether hard to reach groups need further specific services or education in harm reduction.

4.2. Results

4.2.1. Past and present coinfections

The mean (SD) age for HIV cases with no coinfection, was 41 (12) years and 61% were males. The mean (SD) log viral load was 3.33 (1.5) with a mean (SD) of CD4 cell count of 444 (335) cells/mm^3. Most of the non coinfected patients were in stage 1 (51%) or 2 (26.5%) of the WHO classification of HIV at the time of diagnosis.

The hypothesis is that coinfection with TB, HCV or HBV might be associated with reduced $CD4^+$ cell counts or later stage presentation, either as a cause or effect. This is examined in this section. TB coinfection was present in 15% of the whole cohort (208/1383). Most of those patients were males 82% (172/208), with a significant predominance of men compared to non TB coinfected patients in the cohort (P=0.002, X^2 9.2, OR 1.7, 95% CI 1.2-2.6). There was no substantial difference in stage, CD4+ counts or HI viral load at presentation in the TB or non-TB groups (Table 4-1)

		TB coinfection	No TB coinfection
Age mean (SD)		42 (12)	41 (12)
Gender	Male	172/208 (82.3%)	854/1175 (72.7%)
	Female	36/208 (17.3%)	321/1175 (27.3%)
Missing data		None	
Baseline CD4 mean (SD)		402 (362)	444 (335)
Baseline viral load log mean (SD)		3.44 (1.5)	3.61 (1.5)
WHO Stage at presentation	1	113/208 (54%)	706/1175 (60%)
	2	74/208 (35.5%)	367/1175 (31.3%)
	3	20/208 (9.6%)	98/1175 (8.4%)
	4	1/208 (0.4%)	4/1175 (0.3%)

Table (4-1) Comparison of key clinical data in groups with and without TB in this study (n=1383)

Hepatitis C (HCV) tests were performed at least once in about 80% (1101/1383) of the whole cohort. 7.4% (82/1101) were anti-HCV antibody positive, of whom 90% (76/82) were males. This was highly significant compared to non HCV positive patients. ($P < 0.005$, X^2 147, OR 33.4, 95% CI 14.3-77.6). There was no substantial difference in stage, CD4+ counts or HI viral load at presentation in the HCV coinfected patients or non-HCV coinfected groups (Table 4-2)

		HCV coinfection	No HCV coinfection
Age mean(SD)		41 (11)	40 (12)
Gender	Male	76/82 (92.6%)	739/1019 (72.5%)
	Female	6/82 (7.4%)	280/1019 (27.5%)
Missing data		282/1383 (20%)	
Baseline CD4 mean (SD)		415 (306)	444 (337)
Baseline viral load log mean (SD)		4.02 (1.48)	3.32 (1.51)
WHO Stage at presentation	1	49/82 (59.8%)	590/1019 (57.9%)
	2	29/82 (35.4%)	333/1019 (32.7%)
	3	4/82 (4.8%)	92/1019 (9%)
	4	0	4/1019 (0.4%)

Table (4-2) Comparison of key clinical data in groups with and without HCV in this study (n=1101, missing for 282/1383=20%)

Hepatitis B serology was missing for 19% (262/1383) of the whole cohort. 5.3% (59/1121) of patients were HBsAg positive, predominantly males 86.4% (51/59), significantly different from the rest of the cohort (P-value 0.02, X^2 5.04, OR 0.43, 95% CI 0.2-0.91). There was no substantial difference in stage, CD4+ counts or HI viral load at presentation in the HBV coinfected patients or non-HBV coinfected groups (Table 4-3)

HBV coinfected patients had a mean age of 42 years and mean CD4+ of 396 with a viral log of 3.82 WHO staging results were not different from non coinfected patients or from other coinfected patients.

		HBV coinfection	No HBV coinfection
Age mean(SD)		44 (11)	42 (12)
Gender	Male	51/59 (86.4%)	778/1062 (73.3%)
	Female	8/59 (13.6%)	284/1062 (26.7%)
Missing data		262/1383 (19%)	
Baseline CD4 mean (SD)		396 (249)	446 (350)
Baseline viral load log mean (SD)		3.8 (1.2)	3.3 (1.5)
WHO Stage at presentation	1	36/59 (61.1%)	616/1062 (58%)
	2	19/59 (32.2%)	351/1062 (33%)
	3	4/59 (6.7%)	91/1062 (8.5%)
	4	0	4/1062 (0.5%)

Table (4-3) Comparison of key clinical data in groups with and without HBV in this study (n=1062, missing for 262/1383=19%)

4.2.2. IDU and HCV infection:

Hepatitis C (HCV) tests were performed in about 80% (1101/1383) of the whole cohort, of those patents 7.5% (83/1101) were IDU, and 25.3% (21/83) of IDU patients were HCV antibody positive, with a significant statistical different compared to non HCV IDU patients (P<0.05, X^2 41.5, OR 5.3, 95% CI 3-9.2).

		HCV	
		positive	Negative
IDU	Yes	21/83 (25.3%)	62/83(74.7%)
	No	61/1018(6%)	957/1018 (94%)

Table (4-4) IDU and HCV coinfection

4.2.5. TB coinfection and IDU:

1.7% (23/1383) patients were coinfected with TB and were also IDU. Those were 11% of the TB coinfected patients and about 24% of the IDU patients.

There was a significant statistical difference increase in TB coinfection in IDU compared to non-IDU (P<0.01, RR 1.76, 95% CI 1.13-2.41).

The coinfection rates and key risk factors are summarized in table (4-5).

Co-infection rates and key risks				
		IDU	Prison	MSM
Coinfection	Overall prevalence	RR (95% CI) P value	RR (95% CI) P value	RR (95% CI) P value
TB	208/1383 (15%)	1.67 (1.13-2.41) 0.01	1.83 (1.18-2.85) 0.01	1.2 (0.92-1.6) 0.16
HCV	82/1101 (7.4%)	4.22 (2.71-6.57) <0.05	1.94 (1.04-3.63) 0.07	3.1 (1.9-5.04) 0.00
HBV	59/1121 (5.3%)	1.89 (0.93-3.85) 0.08	2.38 (1.25-4.54) 0.01	0.62 (0.27-1.42) 0.23
Syphilis (VDRL)	128/830 (15%)	0.89 (0.41-1.93) 0.4	1.85 (1.04-3.26) 0.03	1.8 (1.3-2.5) 0.000
MSM	264/1026 (26%)	1.03(0.6-1.6) 0.5	1.08 (0.6-1.8) 0.4	---------- ----------

Table (4-5) Summary of Coinfections and key risk factors (TB, N=1383, HCV, N=1101, HBV, N=1121, Syphilis, N=830 and MSM, N=264)

There was 47/264 (17.8%) MSM individuals had been coinfected with TB. 27/209 (13%) of MSM patient had been anti-HCV IgG AB positive where 11/130 (8.5%) of MSM patients were HBsAg positive.

Prisoners	Yes	No	P	X^2	OR	95% CI
TB	24/98 (24.4%)	184/1285 (14.3%)	0.006	7.3	1.9	1.1-3.1
HBV*	8/82 (9.8%)	51/1039 (4.9%)	0.058	3.5	2.1	0.9-4.5
HCV**	7/79 (8.8%)	75/1022 (7.3%)	0.6	0.24	1.2	0.5-2.7
STI	14/98 (14.3%)	74/1285 (5.8%)	0.0008	11.1	2.7	1.4-5

Table 4-6 Prisoner as a risk factor for TB, HBV, HCV and STI (N=98) *N=82, HBV missing data=16 ** N=79, HCV missing data=19 and prisoners=98)

MSM	Yes	No	P	X^2	OR	95% CI
TB	47/264 (17.8%)	161/762 (21.1%)	0.24	1.3	0.8	0.6-1.15
HBV*	5/225 (2.2%)	46/801 (5.7%)	0.03	4.6	0.37	0.1-0.9
HCV**	26/221 (11.7%)	50/805 (6.2%)	0.0005	7.8	2	1.2-3.3
STI	22/264 (8.3%)	66/762 (8.6%)	0.86	0.02	0.95	0.5-1.5

Table 4-7 MSM as a risk factor for TB, HBV, HCV and STI (N=88) *N=51, HBV males ** N=76, HCV males and MSM 264)

4.3. Discussion

TB coinfection rates were expected to be high and 208 (15%) of the patients in this HIV positive cohort found to have active TB co infection. A study published in 2010 have shown that the incidence rate of TB coinfection in HIV positive individuals is at least 30 times higher than it is in the general Saudi population (Al Rajhi et al 2010). TB co infection was significantly more common in males and this is also expected in Saudi society where males are at higher risk for TB infection than females (Al Rajhi et al 2010). However, data collected for HIV/AIDS patients in the year 2012-2013 by the Ministry of Health showed that TB coinfection in HIV/AIDS Saudi patients is 2.16% (UNAIDS 2014). These discrepancies are hard to explain, but emphasize the need for a more systematic approach in both the TB and HIV national programmes, as HIV infection is a very important risk factor both for itself and for drug resistant forms of TB (Bruchfeld, Correia-Neves & Källenius 2015). This is particularly relevant in Jeddah, where this study shows significant association of TB coinfection with IDU and/or being in prison. These hard to reach groups may have poor access to treatment and more difficulty in adhering to treatment and prevention programmes once they have enrolled.

As discussed in chapter one (Table 1-9), the diagnosis of chronic HBV infection can be established based on serum serological and virological markers of HBV. In addition biochemical and histological markers are also used.

Histological evaluation of liver biopsy specimens is a sensitive and accurate indicator of liver disease, however, liver biopsy is an invasive procedure and was reserved for only a few patients (data not shown) (Abaalkhail et al. 2014). Therefore, it is not possible to comment on the severity of liver disease in coinfected patients in this study.

HIV infection increases the risk of liver cirrhosis in HBV patients. In several cohort studies, the risk of liver-related mortality has been found to be 2-3 times higher in HIV/HBV-coinfected patients than in HIV positive individuals with no coinfection (Group 2006).

HIV accelerates HBV viral infection leading to liver damage, so HIV infection significantly increases hepatitis morbidity and mortality. However, most studies have not found HBV coinfection to have a substantial impact on HIV virological responses to ART or on the development of AIDS-defining illness or HIV-related death (Konopnicki et al. 2005; Piroth et al. 2015).HBV coinfection does not appear to influence the rate of HIV progression but may be a surrogate for factors associated with HIV seroconversion. Patients receiving HIV treatment should receive fully active HBV treatment as well. Monotherapy is not recommended while it is recommended to give fully active ART

in conjunction with HBV therapy, as there are limited options for effective HBV treatment thats lack any anti-HIV activity (UCSF 2010). Recent data from West Africa confirm that inadequate HBV cover as part of ART for HIV results in increase of resistant HBV in coinfected patients (Geretti et al. 2010)

In this study patients were only screened for hepatitis B infection by looking for HBsAg. This may occasionally be false negative in some patients and HBV PCR is required (detailed in chapter 1 table 1-9) and this is a limitation of the clinic performance and of the study. Further, because antiHBc or other antigens results were not available, this study can only show rates of current coinfection rather than report rates of past infection with hepatitis B, and thus the lack of serology limits interpretation of exposure risks.

Coinfection with HCV was found in 82/1101 (7.4%) patients, most of whom were men. This may be explained because patients most at risk are IDU especially if they share needle, and MSM. This was supported by a strong link between IDU and HCV in this study. On the other hand HBV co infection was found in only 59 (4%) of the Saudi HIV/AIDS patients. HBV co infection is expected to be lower than HCV co infection in Saudi Arabia, because most HBV transmission occurs in childhood with a different epidemiological pattern then acquiring HIV later in life, when HCV infection is also more likely.

The risk of progression to HCV related liver disease is increased in HIV/HCV coinfected people, with more likely to progress to fibrosis and poor treatment response. In addition, HCV clearance is likely lower than in HIV negative individuals and coinfected individuals have higher HCV viral loads and weaker immune responses against HCV (Sterling et al. 2010).

Sterling and colleagues from Virginia Commonwealth University examined paired liver biopsies from 56 HIV/HCV coinfected patients and matched participants with HCV alone. Samples from coinfected patients showed more necrosis and inflammation. Although the frequency of fibrosis progression was statistically similar, coinfected individuals were twice as likely to progress by two stages (Sterling et al. 2010).

There is evidence that ART in HIV patients can slow hepatitis C progression and should be recommended for HCV/HIV coinfected persons, including those with liver cirrhosis. Therefore, regardless of CD4 cell count it is recommended to start ART for HCV/HIV coinfection, while recognising the possibility of drug interactions (NAT 2012).

HCV genotype has a large effect on the outcome of HCV therapy in HIV/HCV coinfected patients and similar to HCV mono-infected patients. Medrano et al studied the annual prevalence of HCV genotypes/subtypes and their influence on HCV clearance with ART in cohort of HIV/HCV

coinfected patients in Madrid since 2000. HCV genotypes were as follows: 57.1% HCV-1, 1.3% HCV-2, 25.4% HCV-3 and 15.9% HCV-4. They also found that the prevalence of HCV-1 and HCV-4 has increased over the last decade in HIV/HCV-coinfected patients, whereas conversely it has declined for HCV-3, in association with the wider use of HCV therapy (41%) in this population (Medrano et al. 2011).

In our study diagnosing HCV depended on only anti HCV antibody serology which may be false negative in up to 10% of HIV positive patients, so HCV PCR is also indicated. This was a limitation of the clinic performance and probably led to underestimation of the number of patiets with HCV/HIV coinfection in Jeddah.

Stricter guidelines are required in the clinics as only 77% of patients had been tested for HBV and only 80% for HCV at the same stage, although this should be standard practice (BHIVA 2013).

Those found to be HCV positive should have further investigation initially.

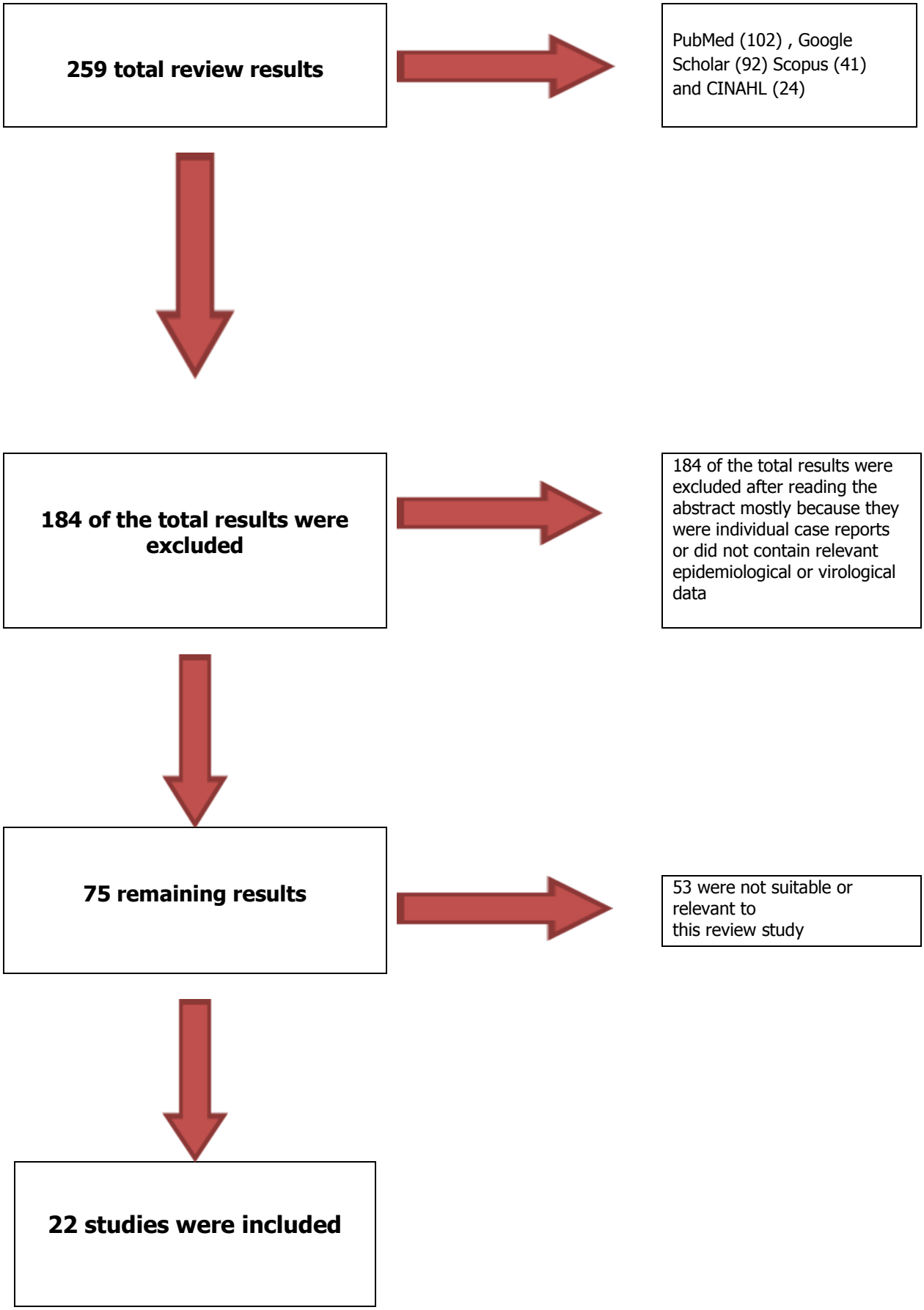
Finally HIV management guidelines globally recommend that all HIV positive people should be tested for hepatitis A, B and C, and be vaccinated against hepatitis A and B if not already immune (BHIVA 2013).

Chapter 5

Antiretroviral resistance in the Region

A systematic literature review was performed of large databases (PubMed, Scopus, Google scholar and CINAHL) using a wide search strategy for the period 2000 to 2014. Combinations of keywords included HIV, AIDS, drug misuse, ART, ARV, coinfections, TB, hepatitis, HIV treatment naïve, resistance together with geographical terms such as Middle East, Saudi Arabia and each country in the Middle East and North Africa (MENA) WHO Region. The WHO website and UNAID websites were also consulted for any reference to HIV and to ART resistance. All relevant papers and reviews were obtained and the reference lists were consulted to identify further sources. Regional scientific journals were also reviewed including the *Saudi Medical Journal, Annals of Saudi Medicine and Journal of Infection and Public Health*. The review started at the beginning of work and was repeated in March 2015 to include the latest publications.

259 results were found in the following databases: PubMed (102), Google scholar (92), Scopus (41) and CINAHL (24). 184 of the total results were excluded after reading the abstract, leaving 75. 53 were not relevant to this study, leaving 22 papers that were included (Table 5-1)


259 total review results
PubMed (102) , Google Scholar (92) Scopus (41) and CINAHL (24)
184 of the total results were excluded
184 of the total results were excluded after reading the abstract mostly because they were individual case reports or did not contain relevant epidemiological or virological data
75 remaining results
53 were not suitable or relevant to this review study
22 studies were included

Country or region	Year of publication	Authors
Developing countries	1998	Palella et al
Developing countries	2004	Moatti et al.
Developed countries	2004	Mocroft et al.
Developed countries	2005	Sabin et al.
Developed countries	2005	Vella et al
Developing countries	2007	Shekelle et al.
Europe and United States	1996	Yerly et al.
Europe	2000	Paredes et al
India	2008	Lall et al.
India	2011	Deshpande er al.
Iran	2014	Baesi et al.
Global	2006	Young et al
Oman	2004	Al Dhahry et al.
Romania	2013	Temereanca et al
Saudi Arabia	2010	Badahdah
Saudi Arabia	2010	Jamjoom et al.
Saudi Arabia (Paediatric)	2011	Al Hajjar et al.
South Africa	2009	Orrell et al
Turkey	2013	Sayan et al.
United States	2005	Hammer
United States	2005	Novak et al.
United States	2008	Huang et al.

Table (5-1) HIV publications used in this study

5.1. ART resistance in developing countries:

Antiretroviral transmitted drug resistance is an important issue all over the world, but there are variations in prevalence different and pattern in countries and regions.

A number of studies have described prevalence rates of transmitted drug resistance (TDR). They range from 0-25 % in different countries. It is as high as 12.9% in North America and 10.9% in Europe and lower in Latin America (6.3%), Africa (4.7%), and in Asia (4.2%)(Frentz, Boucher & Van De Vijver 2012).

With the development of international collaborations, patients with HIV/AIDS patients can nowadays access antiretroviral medications in many developing countries more easily than in the past. It has been estimated that 13 million HIV/AIDS patients were on HAART a decade ago (Young et al. 2006). In 2010, about 5 million HIV positive individuals were receiving ART in Sub-Saharan Africa which represented a 20% increase in ART coverage compared to 2009 (Abo et al. 2015). At the same time, more HIV patients are at risk of developing resistance to ART. This includes those in developing countries, which in turn may lead to an increase in acquired drug resistance in developed countries (Palella et al. 1998)

The problem is increasingly recognised in developing countries that have expanded ART access in recent years, and is especially significant where the supply of antiretroviral drugs (ARVs) is intermittent (Palella, Delaney et al. 1998). While HIV drug-resistance testing is not available in routine care in many resource-limited settings, large surveillance programmes are on-going to monitor regional trends and inform management strategies.

ART resistance was more common when patients were taking single or dual antiretroviral agent therapy (Paredes, Mocroft et al. 2000). More recently, although ARV's have been improved and are easier to take with simpler dosage regimens, the development of resistance is still a major challenge in the management of HIV (Mocroft, Ledergerber et al. 2004; Sabin, Hill et al. 2005).

In addition, ARV resistant virus may transmit to patients with treated non-resistant HIV, or to ART naïve patients. Earlier reports showed that up to 30% of patients in Europe or United States might acquire a virus with drug resistance conferring mutations (Yerly, Rakik et al. 1996). In developed countries, Vella and Palmisano found that about 10% of the naïve patients who started on ART for the first time had

developed ART resistance before they started on the treatment (Vella, Palmisano 2005)

In the USA several studies on ART resistance in treatment naïve HIV/AIDS patients showed resistance to different ART classes. A study of resistance to ART in treatment naïve patients in 2005 by Novak et al showed that resistance for NRTI was present in 7.8% while resistance to NNRTI was present in 3% and to PI in 0.7% (Novak, MacArthur et al. 2005). In 2008 Huang et al found that 9.8% of HIV treatment naïve patients had resistance to NRTI, 4.5% had resistance to NNRTI and about 1.8% showed resistance to PI (Huang, Daar et al. 2008). These two studies showed that the resistance to ART in HIV/AIDS treatment naïve patients had increased over short period between from 2005 to 2008.

Moatti et al. showed in their study in 2004 that ARV drug resistance was also problem in developing countries, although it was then more common in developed countries. To avoid this problem developing the relationship between adherence and ART needed to be addressed. They emphasised the strong link between poor adherence to ART and the increasing likelihood that the patient would develop resistant HIV (Moatti, Spire et al. 2004).

The availability of ART resistance testing is limited in developing countries, potentially leading to a serious problem in management of HIV patients in these countries. Moreover increasing ART resistance in the developing countries may result in acquired ART resistance being transmitted to HIV/AIDS patients in the developed countries (Hammer 2005). Limited data from some studies about ARV resistance in addition to WHO reports from developing countries, have already showed an increase in ARV resistance in these countries.

Two studies were done in India in 2008 and 2011. Lall et al tested newly diagnosed treatment naïve patients in Pune western India in 2008. Resistance to NRTI was present in 5% and resistance to NNRTI and to PI was present in 2.5% each (Lall et al. 2008). In 2011 Deshpande et al. performed genotypic resistance tests on 68 treatment naïve patients in Mumbai, India. No resistance was reported for NRTI but resistance mutations were found to NNRTI in 5.8% and about 3.8% of patients had resistance to PI (Deshpande, Karki, er al. 2011). Temereance et al performed genetic resistance testing on 61 treatment naïve Romanian patients in 2013 and found that 13.1% had resistance to NRTI, 3.3% showed resistance to NNRTI and about 1.6% had resistance mutations to PI (Temereanca., Ene et al 2013).

ARVs have available in Europe and North America for many years and patients can easily access treatment. This may explain the higher prevalence of TDR in developed countries. In Africa, different patterns of resistance to antiretroviral drug classes were seen than in other parts of the world. On the past 5 years there has been an increase in TDR in continent. This may be explained by the wider availability of antiretroviral drugs following efforts by the WHO and international AIDS groups, complicated by intermittent availability of ART at clinic level. In a study in South Africa, Orrel et al tested for ART resistance in 2009 and they found that for 120 HIV patients resistance was minimal: no resistance was recorded for PI, only 1.7% resistance to NNRTI and 3.3% resistance was documented to NRTI (Orrell., et al 2009).

In addition, the differences in TDR rates worldwide, developing countries and developed countries can partially be explained by the use of different methods to define drug resistance. More international efforts and surveillance is needed to monitor the circulating HIV TDR rates and to ensure that ART is adjusted to take into account the evolution of drug resistance (Frentz, Boucher & Van De Vijver 2012)

Country	Author	year	Sample size	Resistance mutations (%)		
				NRTI	NNRTI	PI
Greece	Skoura., Metallidis., et al	2013	238	14.3%	18.9	1.2%
India (West, Pune)	Lall., et al	2008	40	5%	2.5%	2.5%
India (Mumbai)	Deshpande, Karki. et al	2011	68	0	5.8%	3.8%
Romania	Temereanca., Ene et al	2013	61	13.1%	3.3%	1.6%
South Africa	Orrell., et al	2009	120	3.3%	1.7%	0
Turkey	Sayan et al. 2013	2013	117	4.2%	1.7%	1.7%
USA	Novak., MacArthur., et al	2005	491	7.8%	3%	0.7%
USA	Huang., Daar et al	2008	228	9.8%	4.5%	1.8%

Table (5-2) ART resistance reported in HIV treatment naïve patient in selected countries

5.2 ART resistance the Region and KSA

ART resistance is a rising medical problem for HIV/AIDS patients worldwide. In the region of Saudi Arabia the lack of sufficient data for ART resistance is critically affecting the future of patient management. At the regional level only few studies have been published. These studies were heavily biased regarding patient selection. Therefore the current prevalence and patterns of ART resistance in KSA are unknown. This information is essential for defining appropriate therapies for HIV in the Kingdom, especially as resistance may emerge in neighbouring countries (Shekelle et al. 2007).

Al Dhahry et al published the first ART resistance study in the Region in 2004. Blood samples were collected from 93 HIV positive patients from Oman (April - July 2001) for ART resistance testing. Tests were successfully processed for 47/51 treatment naïve patients of whom 3/47 (6.4%) had resistance mutations to reverse transcriptase inhibitors (RTI) and 6/47 (13%) showed resistance to protease inhibitors (PI). Treatment experienced patients showed higher resistance to ART as expected. 24/32 (75%) had resistance to RTI and 7/32 (21.8%) showed resistance mutations to PI (Al Dhahry, Scrimgeour et al. 2004). The study showed the resistance rate for RTI in common but did not define the resistance to NRTI or NNRTI specifically.

There were no other ART resistance publications in the Region until 6 years later when Jamjoom et al tested 63 treatment experienced Saudi patients for ART resistance (2004 - 2009). 26/63 (41%) had mutations conferring resistance to NRTI, 10/63 (16%) showed resistance to NNRTI and 8/63 (13%) had resistance to PI. 2/63 (3%) had resistance to all three ART class (NRTI, NNRTI and PI). The study was performed in Jeddah at King Abdulaziz University and selected only treatment failure patients; no data were available for treatment naïve patients (Jamjoom, Azhar et al. 2010).

Al Hajjar et al in 2011 reviewed genotypic resistance test results for 22 Saudi HIV positive children at a tertiary care centre in Riyadh. They

collected data retrospectively (July 2006 to January 2009). All 22 children were treatment failures and had HIV RNA > 1000 copies/mL. Resistance to any drug was present in 86.4%. There were 24 mutations in protease coding region and 14 in the reverse transcriptase (RT) coding region. M184V was detected in 70% of the isolates (Al Hajjar, Frayha & Althawadi 2011). This study showed high resistance rate in ART failing children in Saudi Arabia.

All these studies were heavily biased regarding patient selection, as they predominantly included patients who had failed one or more switches of ARV therapy. As would be expected, there was a high prevalence of resistance to NRTI, NNRTI and PIs in these studies. ART has been available for over 10 years, but treatment failures are sometimes observed due to drug resistance (either primary or acquired) and problems with treatment adherence. Access to resistance testing has only become available recently and tends to be used only for patients failing therapy (Jamjoom, Azhar et al. 2010). In order to improve clinical care and to improve treatment outcome, ART resistance testing was highly recommended. In addition standard ART genotypic resistance testing should be considered in all HIV positive patients including treatment naïve and at diagnosis.

The current prevalence and patterns of resistance in ART-naïve and treated patients in the Region and in Saudi Arabia are unknown. This information is essential for defining appropriate first-line and salvage

therapies for HIV in the Kingdom for the future, especially as especially as ART resistance testing is not yet routinely available and resistance may emerge in neighbouring countries (Shekelle, Maglione et al. 2007; Bawah, Bongaarts et al. 2006; Moore, Beadsworth et al. 2010).

For example, a recent Turkish study by Sayan et al. tested 117 treatment naïve patients for ART resistance. The samples were collected from June 2009 to February 2012. Resistance to NRTI was found in 5/117 (4.2%), resistance to NNRTI was found in 2/117 (1.7%) and PI resistance was found in in 9/117 (1.7%) (Sayan et al. 2013).

Baesi et al. published a study in 2014 of ART resistance in both treatment naïve and treatment experienced patients Iran. They collected samples from 92 patients over a one year period (2013). 30 patients were treatment naïve and 62 had been taking ART. Results for the treatment naïve patients showed 2/30 (6.7%) resistance to NNRTI and 4/30 (13.3%) had mutations conferring resistance to PI. Results for treatment experienced patients showed that 36/62 (58%) had resistance to treatment; 7/62 (11.3%) had resistance to one class of ART, 27/62 (43.5%) had resistance to two classes of ART and 2/62 (3.2%) had resistance to all three classes (Baesi et al. 2014).

Therefore, this is the first study in the Kingdom and in the whole Region to look for the prevalence and types of ART resistance in treatment naïve HIV patients. This will inform the Saudi authority in the Saudi Ministry of Health (MOH) and the Saudi programme of HIV/AIDS about appropriate first and second line ARV regimens.

Country		**Oman**		**KSA**				**Turkey**	**Iran**	
Reference		(Al Dhahry et al. 2004)		(Jamjoom et al. 2010)		(AlHajjar et al.2011)		(Sayan et al. 2013)	(Baesi et al. 2014)	
Study period		April - July 2001		Aug. 2004 – Jun 2009		July 2006 - January 2009		June 2009 – Feb. 2012	One year (2013)	
Number of patients		93		63		22 (Paediatrics)		117	92 (including IDU patients)	
Treatment Experience		Naïve	ART	Naïve	ART	Naïve	ART	Naïve	Naïve	ART
		51	37 (current) 5 (stopped)	0	63	0	22	117	30	62
Treatment regimen	One	-	3/37 (8%)	-	-	-	3/22 (13%)	-	-	36/62 (58%)
	Two	-	0	-	-	-	5/22 (22%)	-	-	-
	Three	-	5/37 (13.5%)	-	-	-	14/22 63%	-	-	-
Resistance	RTI	3/47 (6.4%)	24/32 (75%)	-	36/63 (57%)	-	-	7/117 (5.9%)	2/30 (6.7%)	-
	NRTI	-	-	-	26/63 (41%)	-	-	5/117 (4.2%)	-	-
	NNRTI	-	-	-	10/63 (16%)	-	19/22 (86.4%)	2/117 (1.7%)	2/30 (6.7%)	-
	PI	6/47 (13%)	7/32 (21.8%)	-	8/63 (13%)	-	-	9/117 (1.7%)	4/30 (13.3%)	-
Resistance by class	One	-	-	-	-	-	-	-	-	7/62 (11.3%)
	Two	-	-	-	-	-	-	-	-	27/62 (43.5%)
	Three	-	-	-	2/63 (3%)	-	-	-	-	2/62 (3.2%)

Table (5-3) – Summary of all ART resistance studies in the Region

Chapter 6

Antiretroviral resistant in treatment naïve patients

6. ART resistance in Jeddah

6.1 Introduction

The introduction of combination antiretroviral therapy revolutionised the management of HIV patients (Palella et al. 1998; Flepp et al. 2001).

Today, patients with HIV should expect a similar life expectancy to HIV negative individuals if their diagnosis is made early and the viral load is well controlled (May et al. 2014). Key factors are the availability of ART, acceptance and treatment adherence by patient, influenced by side-effects (Romanelli & Pomeroy 2000).

The number of HIV patients with "treatment emergent resistance" will increase if an inadequate regimen is chosen or adherence to therapy is poor (Romanelli & Pomeroy 2000). "Primary resistance" may occur in an ART naïve-patient to whom resistance virus is transmitted.

Most international guidelines for HIV treatment programmes include assessment of the presence of resistance in all patients prior to therapy and regular monitoring of HIV viral load during therapy to detect treatment failure early (Johnson et al. 2006). The possibility of resistance would be investigated with molecular methods to characterise the virus for resistance patterns.

In choosing the first choice ART for HIV treatment programme and for description of the likely "standard" regimen for treatment failure patients, it is essential to know the presence of primary ART resistance and the types of secondary resistance that are found. Antiretroviral therapy has been readily available in the Kingdom since the late 1990's, but no studies of primary resistance have been performed in the Kingdom or any other countries in the Region. Only two studies had been published from Oman (Al Dhahry et al. 2004) and KSA (Jamjoom et al. 2010) of resistance in patients who have failed multiple ART combination; they showed high resistance rates.

6.2 Aims

The aim of this study was to determine the prevalence and the pattern of ART resistance in ART naïve patients attending the largest HIV treatment centre in the Kingdom of Saudi Arabia, in Jeddah.

6.3 Methods

These have been discussed in detail on page 124. In brief, sequentially presenting ART naïve patients were identified in the Jeddah clinic between November 2013 and February 2014, and instructed to give an extra sample of blood, which was used for genotypic resistance testing by Sanger and Next Generation Sequencing methods in the UK.

6.4. Demographic details

Over the period of 4 months (November 2013 – February 2014) blood samples were collected from HIV treatment naïve cases followed at King Saud Hospital in Jeddah. Out of all 664 patients 117 were treatment naïve patients and of them 109 were eligible to be enrolled in this study (see page 141).

Of the 109 patients, 71 (65%) were male with a median (range) age of 33 (18-59) years. Overall, 52 (48%) had first received their HIV diagnosis within 6 months prior to attending. The demographic and clinical profile of the patients is compared to that of the ART naïve patients identified in the review of the 2010 patient cohort (Chapter 5) in Table (6-1). The prospective cohort was younger and proportionally more women, in keeping with national trends. CD4+cell counts were lower in the prospective group overall, but HIV viral loads were similar. The proportions of gender and the ages of the two patient groups were similar although there were more women in the prospective sample. No further risk factor data were allowed

ART naïve patients		**2010 Cohort**	**2013/14 Cohort**
Sex	male	248/334 (74%)	71/109 (65%)
Age	Median (range)	35 years (18-78 years)	33 years (18-59 years)
	Mean (SD)	37 (11) years	32 (10.6) years
CD4+	Mean (SD)	524 (320)	327 (296) cells/mm^3
HIV Viral load(log)	Mean (SD)	3.8 (1.15)	3.7 (1.6)copies/ml

Table (6-1) comparing our data from 2010 and 2013/14 cohorts

6.5. Genotypic resistance tests.

Of the 109 samples obtained from ART naïve patients, 87 (80%) were successfully amplified and screened for mutations associated with resistance to NNRTI and NRTI, and 93 (85%) were examined for mutations associated with PIs (Sanger method). The most common HIV-1 subtype is C (41.3%) and the second most common subtype was G (19.3%).

Resistance mutations for all 3 classes of antiretroviral agents were successfully examined in 86 (79%) and conversely, no results at all were could be obtained for 23 (21%) samples. All 22 individual patients with at least one potential resistance mutation detected are summarised in Table 6-2.

Resistance to NNRTI was the most common 9/87 (10.3%) . Resistance to NRTI was only found in 1/87 (1.1%). Resistance to PI was found in 6/93 (6.5%). Moreover 4.5% of the samples showed resistance to EFV and NPV. In addition 1.1% of samples showed resistance to 3TC, FTC, DDI and ABC (M184MV).

	NNRTI			NRTI			PI		
Sample #	Mutations	Resistance		Mutations	Resistance		Mutations	Resistance	
KSH101	None			None			L33F minor	yes	
KSH104	None			None			K43T minor	yes	
KSH011	E138EG	yes		None			None		
KSH017	None			None			L10IL minor	yes	
KSH019	V179E	yes		None			None		
KSH002	V90I		No	None			L10IV minor		No
KSH022	None			M184MV	yes		None		
KSH003	E138A	yes		None			A71T minor		No
KSH035	None			None			L10I minor		No
KSH040	V106I		No	None			None		
KSH042	Y188DY		No	None			None		
KSH049	V90I		No	None			None		
KSH057	E138A	yes		None			None		
KSH060	None			None			L10I minor	yes	
KSH063	V1061V		No	None			None		
KSH066	V106M +V179D	yes		None			None		
KSH067	E138A	yes		None			None		
KSH068	E138EG	yes		None			None		
KSH069	K103N + V106I	yes		None			None		
KSH071	None			None			L10IL minor	yes	
KSH073	None			None			A71V minor		No
KSH078	V179IT		No	None			None		
KSH079	V106IV		No	None			None		
KSH084	V179DV	yes		None			L33IV minor		No
KSH085	None			None			L10I minor	yes	
Total	9/87 (10.3%)	9	7	1/87 (1.1%)	1	0	6/93 (6.5%)	6	5

Table (6-2) ART resistance (Sanger) according to ART class

6.6. Resistance to Reverse Transcriptase Inhibitors (RTI)(Sanger):

87 samples from treatment naïve Saudi HIV recently diagnosed patient were successfully sequenced and tested for resistance to reverse transcriptase inhibitors. The majority 70 (80.5%) were sensitive to RTI. Resistance to at least one RTI was noted in 10 (11.5%) of the cases. Some mutations were noted in 3 (3.4%) which are weakly selected by RTI but if present with another mutation (V179D) will cause significant resistance to RTI.

6.7. Resistance to NNRTI and NRTI (Sanger):

Resistance to NNRTI was detected in 9/87 (10.3%) and in 1/87 (1.1%) in NRTI. The majority of samples showed sensitive sequences with no mutation or non-resistant mutations (88.5%).

6.8. Resistance to PI (Sanger):

93 samples were processed successfully and sequenced for resistance. 6 (6.5%) samples showed significant mutations which can cause resistance to PI.

The frequency of different mutations detected and interpretation of their significance is summarised in table (6-3).

NNRTI	Sample	Resistance mutation	N (%)	Resistance to (ARV)
Total 9/87 (10.3%)	KSH003, KSH057, KSH067	E138A	3 (3.4)	RPV
	KSH011, KSH068	E138EG	2 (2.3)	ETR and RPV
	KSH066, KSH084	V179D	2 (2.3)	EVF and NVP
	KSH066	V106M	1 (1.2)	EVF and NVP
	KSH069	K103M	1 (1.2)	EVF and NVP
	KSH019	K179E	1 (1.2)	EVF and NVP
	KSH069	V106I	1 (1.1)	EVF and NVP
NRTI	**Sample**	**Resistance mutation**	**N (%)**	**Resistance to (ARV)**
Total 1/87 (1.1%)	KSH022	M184MV	1 (1.1)	3TC, ABC, DDI and FTC
PI	**Sample**	**Resistance mutation**	**N (%)**	**Resistance to (ARV)**
Total 6/93 (6.5%)	KSH101	L33F	1 (1.1)	FPV/r and NFV
	KSH104	K43T	1 (1.1)	NFV and TPV
	KSH017 and KSH071	L10IL	2 (2.2)	NFV
	KSH085 and KSH060	L10I	2 (2.2)	NFV

Table (6-3) ART resistance and mutations by drug class (Sanger)

6.9. ART resistance by next generation sequencing (NGS)

Following testing for ART resistance by the Sanger sequencing method, 105/109 (96.3%) HIV treatment naïve patient samples were successfully tested for ART resistance by using deep sequencing and next generation sequencing (NGS) techniques.

Resistance to NNRTI was reported in 24/105 (22.9%) of the total samples, while in the original sequencing method resistance to NNRTI was detected in 9/87(10.3%). Mutations that confer resistance to NRTI were found in 6/105 (5.7%) when samples were processed by deep sequencing (NGS). NRTI resistance with the original sequencing technique resistance was found in only 1/93 (1.1%). Minor HIV sequence mutations to PI were noted in 34/105

(32.4%), whereas by using the original sequencing method there were minor mutations in only 6/93 (6.5%) of the samples.

NRTI			
KSH035	T6MT	**KSH034**	K219R
KSH057	M41LM	**KSH018**	T69A,T69N, M184V
KSH105	M184I	**KSH011**	L210R, L210W
Total 6/105 (5.7%)			
NNRTI			
KSH002	V90I	**KSH084**	V179DV
KSH003	V90I, E138A	**KSH105**	V106I, V108I, M230I
KSH019	V179EV,E138A	**KSH104**	V106I, V106M, V108I
KSH040	V106I	**KSH074**	V106I
KSH049	V90IV	**KSH070**	V106I, V179I
KSH057	E138A	**KSH055**	V90I
KSH062	V106I	**KSH053**	V90I
KSH066	V106M, V179D	**KSH052**	E138A
KSH067	E138A	**KSH048**	E138A
KSH069	K103N, V106I	**KSH033**	E138A
KSH078	V179I	**KSH002**	E138A
KSH079	E138A,V106IMV	**KSH019**	E138A
Total 24/105 (22.9%)			
PI			
KSH002	L10LV	**KSH056**	K20I
KSH003	A71T	**KSH060**	K20I, L10I
KSH007	T74S	**KSH061**	K20I
KSH014	T74S	**KSH064**	K20I
KSH018	K20I	**KSH065**	K20I
KSH019	K20I	**KSH071**	K20I, L10IL
KSH025	K20I	**KSH073**	A71V
KSH031	K20I	**KSH074**	K20I
KSH035	L10I	**KSH075**	K20I
KSH037	K20I	**KSH077**	M46I
KSH039	K20I	**KSH078**	K20I, M46I
KSH041	K20I	**KSH079**	K20I, T74S
KSH044	K20I	**KSH080**	K20I
KSH045	K20IK	**KSH084**	L33IV
KSH049	K20I	**KSH085**	K20I, L10I
KSH053	K20I	**KSH088**	T74ST
KSH055	K20I	**KSH089**	T74S
Total 34/105 (32.4%)			

Table (6-4) Summary of all ART resistance mutations (NGS)

	Genotypic Testing (Sanger)					Genotypic testing (NGS 20%)*				
Sample	Subtype	Mutation/ART class			Remarks	Subtype	Mutation/ART class			Remarks
		NNRTI	NRTI	PI			NNRTI (%)	NRTI (%)	PI (%)	
KSH001	C	None	None	None		C	None	None	None	
KSH002	C	None	None	None		C	V90I(84%)	None	L10LV(35%)	
KSH003	C	E138A	None	None		C	E138A(23%)	None	A71T(96%)	
KSH004	C	None	None	None		C	None	None	None	
KSH005	Unable	None	None	None		CRF01_AE	None	None	None	
KSH006	D	None	None	None		D	None	None	None	
KSH007	C	None	None	None		C	None	None	T74S(99%)	
KSH008	C	None	None	None		C	None	None	None	
KSH009	CRF02_AG	None	None	None		CRF02_AG	None	None	None	
KSH010	C	None	None	None		C	None	None	None	
KSH011	Unable	E138EG	None	None		C	None	L210R(2%), **L210W**(2%)	None	
KSH012	Unable	None	None	None		Unable	None	None	None	
KSH013	B	None	None	None		B	None	None	None	
KSH014	A	None	None	None		A	None	None	T74S(35%)	
KSH015	Unable				Not processed	Unable	None	None	None	
KSH016	Unable				Not processed	Unable	None	None	None	
KSH017	Unable	None	None	L10IL		Unable				Not processed
KSH018	G	None	None	None		G	T69A(10%), T69N(7%), **M184V (11%)**	None	K20I(100%)	
KSH019	G	K179E	None	None		G	V179E(57&%), E138A(3.6%)	None	K20I(100%)	
KSH020	C	None	None	None		C	None	None	None	
KSH021	CRF01_AE	None	None	None		CRF01_AE	None	None	None	
KSH022	G	None	**M184MV**	None		G	None	None	None	
KSH023	C	None	None	None		C	None	None	None	
KSH024	C	None	None	None		C	None	None	None	

Table (6-5) comparison between ART resistance by Sanger and NGS methods (n=109)

*20% cut-off was used in NGS to compare results with Sanger methods which had threshold of 20%.

	Genotypic Testing (Sanger)					Genotypic testing (NGS 20%)*				
Sample	Subtype	Mutation/ART class			Remarks	Subtype	Mutation/ART class			Remarks
		NNRTI	NRTI	PI			NNRTI (%)	NRTI (%)	PI (%)	
KSH025	Unable	Not processed		None		Unable	None	None	None	
KSH026	G	None	None	None		G	None	None	None	
KSH027	A	None	None	None		A	None	None	None	
KSH028	Unable				Not processed	Unable	None	None	None	
KSH029	Unable				Not processed	Unable	None	None	None	
KSH030	C	None	None	None		C	None	None	None	
KSH031	G	None	None	None		G	None	None	K20I(96%)	
KSH032	Unable	None	None	None		A/B	None	None	None	
KSH033	C	None	None	None		C	E138A (21%)	None	None	
KSH034	C	None	None	None		C	None	K219R (3.4%)	None	
KSH035	K	None	None	None		K	None	T69NT (21%)	L10I(100%)	
KSH036	C	None	None	None		C	None	None	None	
KSH037	G	None	None	None		G	None	None	K20I(90%)	
KSH038	C	None	None	None		C	None	None	None	
KSH039	G	None	None	None		G	None	None	K20I(90%)	
KSH040	D	None	None	None		D	V106IV (63%)	None	None	
KSH041	G	None	None	None		G	None	None	K20I(90%)	
KSH042	CRF01_AE	None	None	None		CRF01_AE	None	None	None	
KSH043	Unable				Not processed	Unable	None	None	None	
KSH044	CRF02_AG	None	None	None		CRF02_AG	None	None	K20I(99%)	
KSH045	G	None	None	None		G	None	None	K20IK(70%)	
KSH046	C	None	None	None		C	None	None	None	
KSH047	D	None	None	None		D	None	None	None	
KSH048	C	None	None	None		C	E138A (5.1%)	None	None	

Table (6-5) comparison between ART resistance by Sanger and NGS methods (n=109)

*20% cut-off was used in NGS to compare results with Sanger methods which had threshold of 20%.

	Genotypic Testing (Sanger)					Genotypic testing (NGS 20%)*				
Sample	Subtype	Mutation/ART class			Remarks	Subtype	Mutation/ART class			Remarks
		NNRTI	NRTI	PI			NNRTI (%)	NRTI (%)	PI(%)	
KSH049	G	None	None	None		G	V90IV(63%)	None	K20I(99%)	
KSH050	C				Not processed	C	None	None	None	
KSH051	D				Not processed	D	None	None	None	
KSH052	Unable	None	None	None		D/G	E138A(14.6%)	None	None	
KSH053	G	None	None	None		G	V90I (5%)	None	K20I(99%)	
KSH054	Unable	None	None	None		Unable	None	None	None	
KSH055	G	None	None	None		G	V90I(4.8%)	None	K20I(99%)	
KSH056	G	None	None	None		G	None	None	K20I(99%)	
KSH057	C	E138A	None	None		C	E138A(85%)	**M41LM(21%)**	None	
KSH058	Unable	None	None	None		J/D	None	None	None	
KSH059	C	None	None	None		C	None	None	None	
KSH060	CRF02_AG	None	None	L10I		CRF02_AG	None	None	K20I(100%) L10I(99%)	
KSH061	G	Not processed		None		G	None	None	K20I(99%)	
KSH062	D	None	None	None		D	V106I(99%)	None	None	
KSH063	G	None	None	None		G				Not processed
KSH064	G	None	None	None		G	None	None	K20I(99%)	
KSH065	CRF02_AG	None	None	None		CRF02_AG	None	None	K20I(99%)	
KSH066	D	V179D,**V106M**	None	None		D	**V106M(78%)** V179D(88%)	None	None	
KSH067	C	E138A	None	None		C	E138A(99%)	None	None	
KSH068	C	E138EG	None	None		C	None	None	None	
KSH069	D	**K103N**,V106I	None	None		D	**K103N(99%)** V106I(98%)	None	None	
KSH070	D	None	None	None		D	V179I(75%)	**M41LM(22%)**	None	
KSH071	G	None	None	L10IL		G	None	None	L10IL(59%) K20I(99%)	
KSH072	C	None	None	None		C	None	None	None	

Table (6-5) comparison between ART resistance by Sanger and NGS methods (n=109)

*20% cut-off was used in NGS to compare results with Sanger methods which had threshold of 20%.

	Genotypic Testing (original)					Genotypic testing (NGS 20%)*				
Sample	Subtype	Mutation/ART class			Remarks	Subtype	Mutation/ART class			Remarks
		NNRTI	NRTI	PI			NNRTI (%)	NRTI(%)	PI (%)	
KSH073	B		Not processed	None		B	None	None	A71V(99%)	
KSH074	CRF02_AG	None	None	None		CRF02_AG	V106I(6.2%)	None	K20I(94%)	
KSH075	CRF02_AG	None	None	None		CRF02_AG	None	None	K20I(99%)	
KSH076	Unable	None	None	Not processed		C	None	None	None	
KSH077	J	None	None	None		J	None	None	M46I(3.2%)	
KSH078	G	None	None	None		G	V179T(48.5%)	None	K20I(91%) M46I(13.6%)	
KSH079	G	None	None	None		G	**V106I(24%)**	None	K20I(99%) T74S(87%)	
KSH080	G	None	None	None		G	None	None	K20I(99%)	
KSH081	Unable		Not processed	None						Not processed
KSH082	C	None	None	None		C	None	None	None	
KSH083	C	None	None	None		C	None	None	None	
KSH084	D	V179D	None	None		D	V179D(33%)	None	L33IV(53%)	
KSH085	Unable	None	None	L10I		G/K	None	None	L10I(90%) K20I(93%)	
KSH086	C	None	None	None		C	None	None	None	
KSH087	C	None	None	None		C	None	None	None	
KSH088	C	Not processed		None		C		None	T74S(77%)	
KSH089	C	None	None	None		C	None	None	T74S(98%)	
KSH090	C	None	None	None		C	None	None	None	
KSH091	C	None	None	None		C	None	None	None	
KSH092	C	None	None	None		C	None	None	None	
KSH093	Unable				Not processed	Unable	None	None	None	
KSH094	Unable				Not processed	Unable	None	None	None	
KSH095	Unable				Not processed	Unable	None	None	None	
KSH096	C				Not processed	C	None	None	None	

Table (6-5) comparison between ART resistance by Sanger and NGS methods (n=109)

*20% cut-off was used in NGS to compare results with Sanger methods which had threshold of 20%.

	Genotypic Testing (original)					Genotypic testing (NGS 20%)*				
Sample	Subtype	Mutation/ART class			Remarks	Subtype	Mutation/ART class			Remarks
		NNRTI	NRTI	PI			NNRTI (%)	NRTI(%)	PI (%)	
KSH097	C	None	None	None		C	None	None	None	
KSH098	C	None	None	None		C	None	None	None	
KSH099	G				Not processed	G	None	None	K20I(100%)	
KSH100	C				Not processed	C	None	None	None	
KSH101	G	Not processed		L33F		G	None	None	None	
KSH102	C	None	None	None						Not processed
KSH103	C	None	None	None		C	None	None	None	
KSH104	G	None	None	K43T		G	V106I(9%), **V106M**(2%), V108I(7%)	None	K20I(44%)	
KSH105	K	None	None	None		K	V106I(97%) V108I(97%)	**M184I(3%)**	None	
KSH106	C	None	None	None		C	None	None	None	
KSH107	D	None	None	None		D	None	None	None	
KSH108	C				Not processed	C	None	None	None	
KSH109	C				Not processed	C	None	None	None	

Table (6-5) comparison between ART resistance by Sanger and NGS methods (n=109)

*20% cut-off was used in NGS to compare results with Sanger methods which had threshold of 20%.

6.10. Discussion

6.10.1 ART resistance:

Blood samples were collected from 109/116 (94%) patients approached to join the study. 71 (65%) were male and 52 (48%) had been diagnosed with HIV within the last 6 months. HIV RNA was successfully amplified from 96, and sequence data obtained from 93 Prot amplicons and 87 RT amplicons. Mutations at putative resistance sites were detected in 25/96 (26%). Those with significant potential to confer resistance to NNRTI were found in 9/87 (10.3%), with resistance to NRTI in 1/87 (1.1%) and PI in 6/93 (6.5%). 4.5% of the samples showed resistance to efavirenz and nevirapine. In addition, 1.1% of samples showed resistance to lamivudine, emtricitabine, didanosine and abacavir (M184MV).

To confirm and extend findings, the samples were tested using next generation sequencing (deep sequencing technique) to look for more mutations conferring resistance to ART. This is a recently introduced technique which can examine in more depth the nucleic acid sequence and therefore gives a better comparison with the wild type HIV virus.

No studies have been performed in the Region or in KSA on ART resistance using next generation sequencing. This is the first study to look systematically for ART resistance in treatment naïve patients and

to compare the results obtained by using both sequencing techniques (deep sequencing and the original Sanger sequencing technique).

Using NGS genotypic resistance testing, revealed mutations conferring resistance to protease inhibitors (PI) in 34/105 (32.4%) and to reverse transcriptase inhibitors (RTI) in 24/105 (22.9%).

ART resistance Results Summary	
Sanger	NGS*
• Total sample size was 109 samples.	• Total sample size was 105 samples
• Resistance to NNRTI was the most common 9/87 (10.3%)	• Resistance to NNRTI was the most common 24/105 (22.9%)
• Resistance to NRTI was only 1/87 (1.1%)	• Resistance to NRTI was 6/105 (5.7%)
• Resistance to PI was 6/93(6.5%)	• Resistance to PI was 34/105 (32.4%)

Table (6-6) ART resistance Results Summary (n=109)

*20% cut-off was used in NGS to compare results with Sanger methods which had threshold of 20%.

Several protease inhibitors have been introduced for HIV treatment. It is important to know how the effect of each protease inhibitor mutation impacts on resistance to other PI. Boosted PI regimens (often using low dose raltegravir) have been found to be more effective at decreasing viral replication than unboosted protease inhibitor regimens (Rhee et al. 2010)

Protease inhibitors reduce HIV viral replication but some residual viral activity remains. This activity may lead to mutations which produce resistance. In addition, many of the PIs have poor oral bioavailability or short half-lives. This may affect adherence to treatment because doses will be given in higher amount and more frequently. Moreover, the side effects of PIs may be increased by prescribing higher doses. Such toxic effects may include gastrointestinal side effects (diarrhoea), rash, renal colic and dry skin. These factors may further increase the likelihood of inadequate adherence and subsequently induce resistance (Walmsley 2007). Thus, protease inhibitor use can result in high levels of resistance and numerous side effects (Kožíšek et al. 2014).

Other factors may be considered and may affect resistance to ART, including HIV exposure mode, pre-treatment viral load and CD4 cell count, content of first ART regimen, hepatitis B/C co-infection, prior AIDS diagnosis, HIV subtype, and adherence to treatment. Transmitted drug resistance is also significant for drug-specific or drug class–specific resistance to treatment outcome and its significance requires further study.

Sirivichayakul et al recently studied transmitted drug resistance and ART outcomes in non-subtype B HIV-1–infected patients in South East Asia (Sirivichayakul et al. 2014). 1471 patients were included from 12 sites in Thailand, Malaysia, Hong Kong, the Philippines and Indonesia. All patients were ART-naïve. The common subtype in this region is

HIV-1 CRF01_AE. In our study in Saudi Arabia about 8% of patients were HIV 1 subtype CRF02_AG (see chapter 7). Drug mutations that confer resistance for HIV treatment were found in 4.1% before ART initiation. They found that patients taking ART with resistance to least one drug class will have a 3.12-fold higher chance of virological failure compared to those without resistance (Sirivichayakul et al. 2014)

Country		**Oman**		**KSA**					**Turkey**	**Iran**	
Reference		(Al Dhahry et al. 2004)		(Jamjoom et al. 2010)		This study			(Sayan et al. 2013)	(Baesi et al. 2014)	
Study period		April - July 2001		Aug. 2004 – Jun 2009		Nov.2013 –Feb.2014			June 2009 – Feb. 2012	One year (2013)	
Number of patients		93		63		109			117	92	
Treatment Experience		Naïve	ART	Naïve	ART	Naïve		ART	Naïve	Naïve	ART
		51	37 (current) 5 (stopped)	0	63	109		0	117	30	62
Treatment regimen	One	-	3/37 (8%)	-	-	Sanger -	NGS -	-	-	-	36/62 (58%)
	Two	-	0	-	-	-	-	-	-	-	-
	Three	-	5/37 (13.5%)	-	-	-	-	-	-	-	-
Resistance	RTI	3/47 (6.4%)	24/32 (75%)	-	36/63 (57%)	10/87 (11.4%)	30/105 (28.6%)	-	7/117 (5.9%)	2/30 (6.7%)	-
	NRTI	-	-	-	26/63 (41%)	(1/87) 1.1%	6/105 (5.7%)	-	5/117 (4.2%)	-	-
	NNRTI	-	-	-	10/63 (16%)	9/87 (10.3%)	24/105 (22.9%)	-	2/117 (1.7%)	2/30 (6.7%)	-
	PI	6/47 (13%)	7/32 (21.8%)	-	8/63 (13%)	6/93 (6.5%)	34/105 (32.4%)	-	9/117 (1.7%)	4/30 (13.3%)	-
Resistance by class	One	-	-	-	-	16/93 17.2	64/105 60%	-	-	-	7/62 (11.3%)
	Two	-	-	-	-	0	4/105 3.8%	-	-	-	27/62 (43.5%)
	Three	-	-	-	2/63 (3%)	0	0	-	-	-	2/62 (3.2%)

Table (6-7) This study results compared to ART resistance studies results in the Region

As expected, more mutations that confer resistance to ART were discovered by NGS sequencing. Resistance to NNRTI was found in 22.9% compared to 10.3% detected using the original sequencing technique. 5.7% mutations that confer resistance to NRTI were found using deep sequencing technique (next generation sequencing) while only 1.1% resistance to NRTI was found using the original technique. The study showed that the rate of minor mutations that may confer PI resistance is 32.4% using the deep sequencing and only 6.5% using the original sequencing.

To put this in context the finding of Sanger and NGS sequencing in this study have been compared with other studies of ART in adults in Table 6-7. Although the numbers in each study are small, there is a lot of similarity in the findings in ART naïve patients in Oman (2004) and Iran (2014) where there was RT resistance in 6.4% and 6.7% respectively, compared to 11.4% in this study in KSA. Similarly, these studies revealed 13% and 13.3% PI resistance respectively, compared to 6.5% this study.

NNRTI resistance was found in 6.7 % of patients in Iran compared to 10.3% in this study.

Thus, there seem to be 5-15% transmitted resistance to each class, with a trend towards higher levels of NNRTI resistance in this more comprehensive study in KSA.

6.10.2 Low dose efavirenz

Efavirenz is one of the most commonly used and recommended NNRTI in first line regimen for HIV treatment naïve patients. Advantages of efavirenz include a low pill burden (once daily), multiple clinical trials have shown good drug potency with few metabolic effects, and it is not known to have long term side effects. On the other hand, efavirenz is associated with increased frequency of transmitted drug resistance, cross class resistance, rash and CNS side effects (Stone et al. 2009).

In 2014 the ENCORE1 study group published a randomised, double-blind, placebo-controlled study comparing the efficacy of low dose efavirenz (400 mg) versus standard 600 mg dose in HIV treatment naïve patients. Participants were recruited from centres in 13 different countries include Argentina, Australia, Chile, Germany, Hong Kong, Israel, Malaysia, Mexico, Nigeria, Singapore, South Africa, Thailand and the UK. The study included 311 patients on reduced 400mg dose and 295 patients on regular 600mg dose followed for 48 weeks. The study showed that a reduced dose of 400 mg efavirenz is non-inferior to the standard dose of 600 mg, when combined with tenofovir and emtricitabine during 48 weeks in ART-naïve adults with HIV-1 infection. In addition reducing the dose will also improve the cost and reduce the side effects (Group 2014)

This result of efavirenz efficacy was also supported by earlier published reports describing the efficacy and potency of efavirenz-containing regimens in first line antiretroviral therapy (Puls et al. 2010).

In Saudi Arabia the most common first line ART prescribed to HIV treatment naïve patents is a combination of tenofovir and emtricitabine in addition to the regular dose of 600mg efavirenz. Dose reduction should be considered in the future because of the reduction of cost and side effects. However, it will be important to monitor the effect of this on acquired resistance, and patients should be tested for TDR, as 4.5% have resistance to EFV and NVP by Sanger sequencing and a higher proportion of 9.5% was detected by NGS method.

6.10.3 Etravirine and rilpivirine Resistance:

Rilpivirine resistance-associated-mutations (RAMs) were found in 5 (5.7%) of treatment naïve HIV patients. Mutations were noted in E138A (3.4%) and E138EG (2.3%). 80% were HIV-1 subtype C. Etravirine only RAMs detected in 2 (2.3%). The mutation noted was E138EG. These drugs have hardly been used in KSA so their presence is a cause for concern.

In 2013 Asahchop and colleagues studied etravirine and rilpivirine resistance in viruses containing NNRTI mutations at base line. The study showed that in wild type viruses E138K or E138G mutations were detected following pressure with etravirine or rilpivirine, prior to

the appearance of other NNRTI resistance mutations. Etravirine and rilpivirine are likely to select for E138K as major RAMs (Asahchop et al 2013)

Geretti and colleagues examined both sensitive methods of testing for of HIV-1 RNA in plasma and Sanger sequencing to detect drug resistance prior to starting first-line ART with etravirine or efavirenz. They found that 13.9% of the patients had polymorphic RAMs to etravirine of V90I, V106I and E138A (Geretti et al 2014)

Gallien and colleagues studied the resistance mutations to rilpivirine and etravirine in successfully treated HIV patients pre-exposed to efavirenz or nevirapine. The study showed RAMs for rilpivirine in 41 (32%) individuals. 6% of the mutations were in E138A/G/K/Q/R/S. Etravirine mutations were detected in 5 (4%) individuals. They concluded that switching to rilpivirine-based regimens should not be recommended (Gallien et al 2014).

These emerging resistances are significant and can probably be generalized over the Saudi Kingdom. Therefore, future plans for Saudi programme for HIV/AIDS fighting should include routine ART resistance test for HIV treatment naïve patients.

6.10.4 Pre-exposure and post exposure prophylaxis

Pre-exposure prophylaxis (PrEP) describes the prescription of an antiretroviral drug for a person who does not have HIV infection, as a strategy for prevention of acquisition of the virus (CDC 2014).

Post-exposure prophylaxis (PEP) with ART has long been recommended for people who experience exposures to HIV through accident, assault, rape, accidental injury, occupational and other sources. This is now well accepted and is a standard of care (Ford et al. 2015), as is perinatal and prenatal prescription of ART to mother and infant to reduce the chance of mother to child transmission of HIV (Hurst et al. 2014). The use of PrEP was a logical extension of these polices for individuals at reported high risk of infection but has been controversial.

It is an optional treatment for people who are HIV negative but with high risk of getting the infection, such as MSM with frequent partner change. Currently, the only drug which any health organization recommends for PrEP is the combination of tenofovir/emtricitabine. Daily oral PrEP with the fixed-dose combination of tenofovir (TDF) 300 mg and emtricitabine (FTC) 200 mg has been shown to be safe and effective in reducing the risk of sexual HIV acquisition in adults.The Centers for Disease Control recommends PrEP as it is a powerful HIV prevention tool and can be combined with condoms and other

prevention methods to provide even greater protection than when used alone (CDC 2014).

The only medication regimen approved by the Food and Drug Administration and recommended for PrEP with all the populations specified in this guideline is daily TDF 300 mg co-formulated with FTC 200 mg (Truvada) (CDC 2014).

However, there are concerns about promoting ART resistance when PrEP is recommended. Daily oral PrEP using emtricitabine/tenofovir (FTC/TDF) or even TDF alone is safe and effective for preventing HIV acquisition by MSM but adherence remains an important factor in resistance development. Moreover this regimen does not achieve sufficient activity for full HIV virus suppression, so PrEP in people already infected with HIV will probably induce drug resistance. Therefore, recommendations for PrEP emphasize the importance of HIV testing prior to starting or restarting PrEP (Grant & Liegler 2015)

Chapter 7

HIV subtypes and phylogenetic analysis

7.1 Virus Clade

At least three separate zoonotic transmissions resulted in the formation of three distinct HIV-1 groups: M (main), O (outlier), and N (non-M/non-O). About 90% of HIV-1 infections are classified as group M and these are distributed worldwide. Group O infections are endemic to several west central African countries and represent 1 to 5% of all HIV-1 infection in those areas. The M group is further divided into different subtypes, the geographical distributions of which are shown in figure (7-1). Almost 50% of global HIV-1 infection is subtype C which is the dominant subtype in Africa, Ethiopia and India. About 12% of HIV infection worldwide is subtype A. Subtype B is most commonly found in Americas, Europe and Australia. Circulating recombinant forms (CRF) are commonly found in East Asia, West and Central Africa, and the Middle East and North Africa regions (Peters et al. 2013).

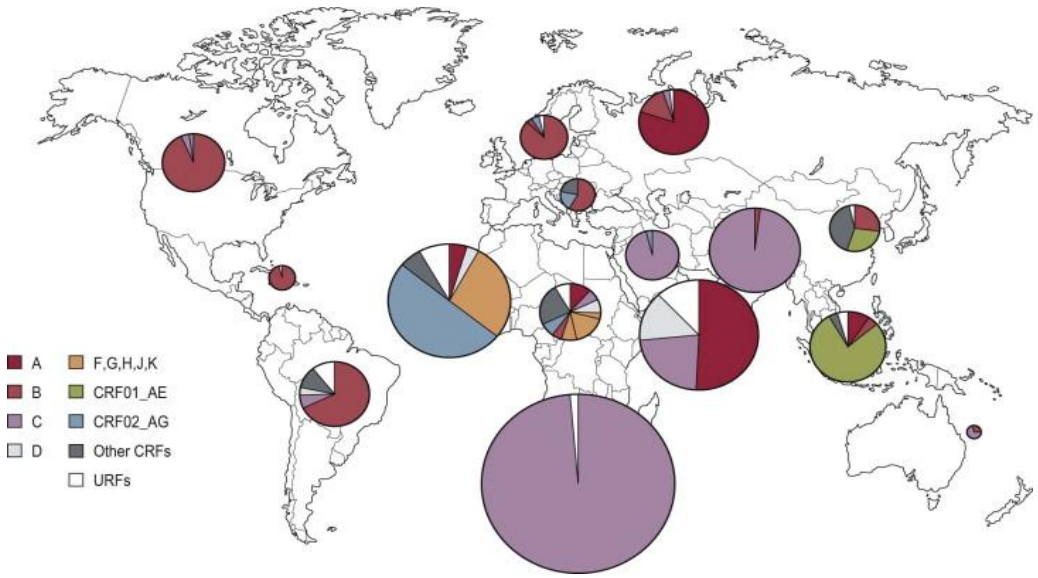


Figure (7-1) HIV-1 subtypes global map (Peters et al. 2013)

In a recent Turkish study, looking at 117 newly diagnosed HIV positive patients, CRFs were the most common subtypes (CRF 02_AG, CRF 01_AE, CRF 12_BF and CRF 03_AB; 47%, 55/117) and B (33.3%, 39/117) (Sayan et al. 2013). A similar study in Iran looking at 50 HIV positive ART naïve patients, revealed that 95.7% of sequenced samples were CRF35_AD, 2.1% and CRF01_AE 2.1% (Jahanbakhsh et al. 2013). As in most studies from Iran, many patients were IDU and there is clearly spread of limited clades of HIV in this population due to syringe sharing.

Previous phylogenetic analysis in Saudi patients showed that the most common HIV-1 subtypes are C (39.3%) G (25%), B (17.9%), D (3.6%), A (1.8%) and CRF02_AG (1.8%) (Badreddine et al. 2007), and similar results were obtained in two other studies in the Kingdom (Jamjoom et al. 2010; Alzahrani 2008).

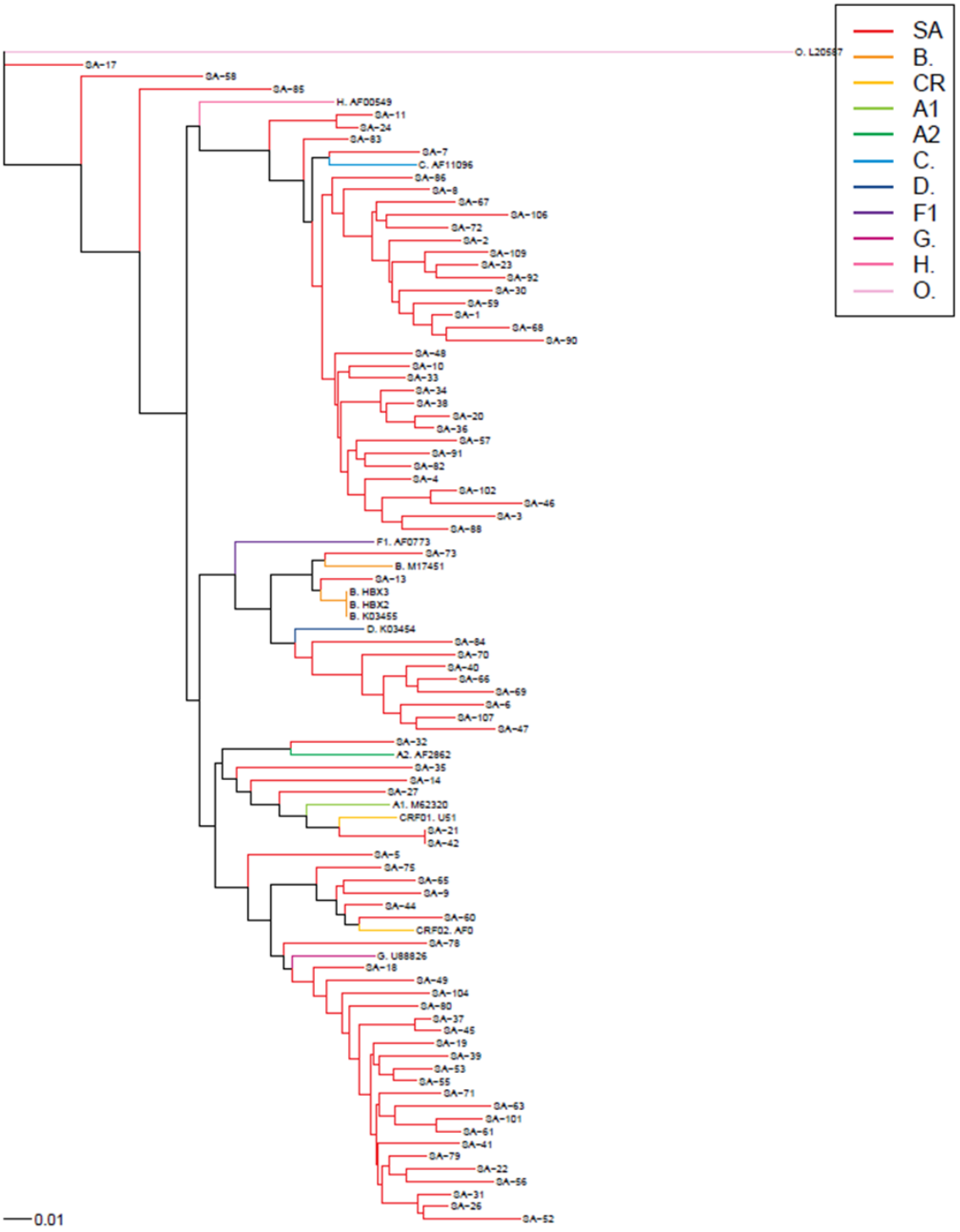
In this study, it was not possible to identify the virus clade in 20 (18%) of the samples. The results of the whole cohort of 109 are detailed in table 7-1 and summarised in Figures 7-2 and 7-3.

The most common clade by Sanger method was C (41/89, 46%), followed by subtype G (23/89, 26%). A cluster of CRF02_A and CRF01_AE was present in (8/87, 9%). Other subtypes included D (10/89, 11%), A (2/89, 2.2%), B (2/89, 2.2%), K (2/89, 2.2%) and J (1/89, 1.2%).

A representative phylogenetic tree derived from sequences is shown in Figure 7.2. This tree illustrates high levels of HIV-1 strain diversity within the Saudi study population.

The most common clade by NGS method was C (42/95, 44.2%), followed by subtype G (23/95, 24.2%). A cluster of CRF02_A and CRF01_AE was present in (9/95, 9.5%). Other subtypes included D (10/95, 10.5%), A (2/95, 2.1%), B (2/95, 2.1%), K (2/95, 2.1%) and J, J/D, D/G, G/K and A/B (1/95, 1.1%).

Thus, the results were very similar by both Sanger and NGS methods (Table 7-1). Results when both were successful were concordant, with 6/109 samples identified by NGS methods but not by Sanger.



(Figure 7-2) HIV-1 subtypes in this study (Sanger method N=109)

Sample	subtype		sample	subtype		sample	Subtype	
	sanger	NGS		sanger	NGS		sanger	NGS
KSH001	C	C	KSH038	C	C	KSH075	CRF02_AG	CRF02_AG
KSH002	C	C	KSH039	G	G	KSH076	Unable	C
KSH003	C	C	KSH040	D	D	KSH077	J	J
KSH004	C	C	KSH041	G	G	KSH078	G	G
KSH005	Unable	CRF01_AE	KSH042	CRF01_AE	CRF01_AE	KSH079	G	G
KSH006	D	D	KSH043	Unable	Unable	KSH080	G	G
KSH007	C	C	KSH044	CRF02_AG	CRF02_AG	KSH081	Unable	Unable
KSH008	C	C	KSH045	G	G	KSH082	C	C
KSH009	CRF02_AG	CRF02_AG	KSH046	C	C	KSH083	C	C
KSH010	C	C	KSH047	D	D	KSH084	D	D
KSH011	Unable	C	KSH048	C	C	KSH085	Unable	G/K
KSH012	Unable	Unable	KSH049	G	G	KSH086	C	C
KSH013	B	B	KSH050	C	C	KSH087	C	C
KSH014	A	A	KSH051	D	D	KSH088	C	C
KSH015	Unable	Unable	KSH052	Unable	D/G	KSH089	C	C
KSH016	Unable	Unable	KSH053	G	G	KSH090	C	C
KSH017	Unable	Unable	KSH054	Unable	Unable	KSH091	C	C
KSH018	G	G	KSH055	G	G	KSH092	C	C
KSH019	G	G	KSH056	G	G	KSH093	Unable	Unable
KSH020	C	C	KSH057	C	C	KSH094	Unable	Unable
KSH021	CRF01_AE	CRF01_AE	KSH058	Unable	J/D	KSH095	Unable	Unable
KSH022	G	G	KSH059	C	C	KSH096	C	C
KSH023	C	C	KSH060	CRF02_AG	CRF02_AG	KSH097	C	C
KSH024	C	C	KSH061	G	G	KSH098	C	C
KSH025	Unable	Unable	KSH062	D	D	KSH099	G	G
KSH026	G	G	KSH063	G	G	KSH100	C	C
KSH027	A	A	KSH064	G	G	KSH101	G	G
KSH028	Unable	Unable	KSH065	CRF02_AG	CRF02_AG	KSH102	C	C
KSH029	Unable	Unable	KSH066	D	D	KSH103	C	C
KSH030	C	C	KSH067	C	C	KSH104	G	G
KSH031	G	G	KSH068	C	C	KSH105	K	K
KSH032	Unable	A/B	KSH069	D	D	KSH106	C	C
KSH033	C	C	KSH070	D	D	KSH107	D	D
KSH034	C	C	KSH071	G	G	KSH108	C	C
KSH035	K	K	KSH072	C	C	KSH109	C	C
KSH036	C	C	KSH073	B	B			
KSH037	G	G	KSH074	CRF02_AG	CRF02_AG			

Table (7-1) HIV -1 Subtypes by Sanger and NGS methods (N=109)

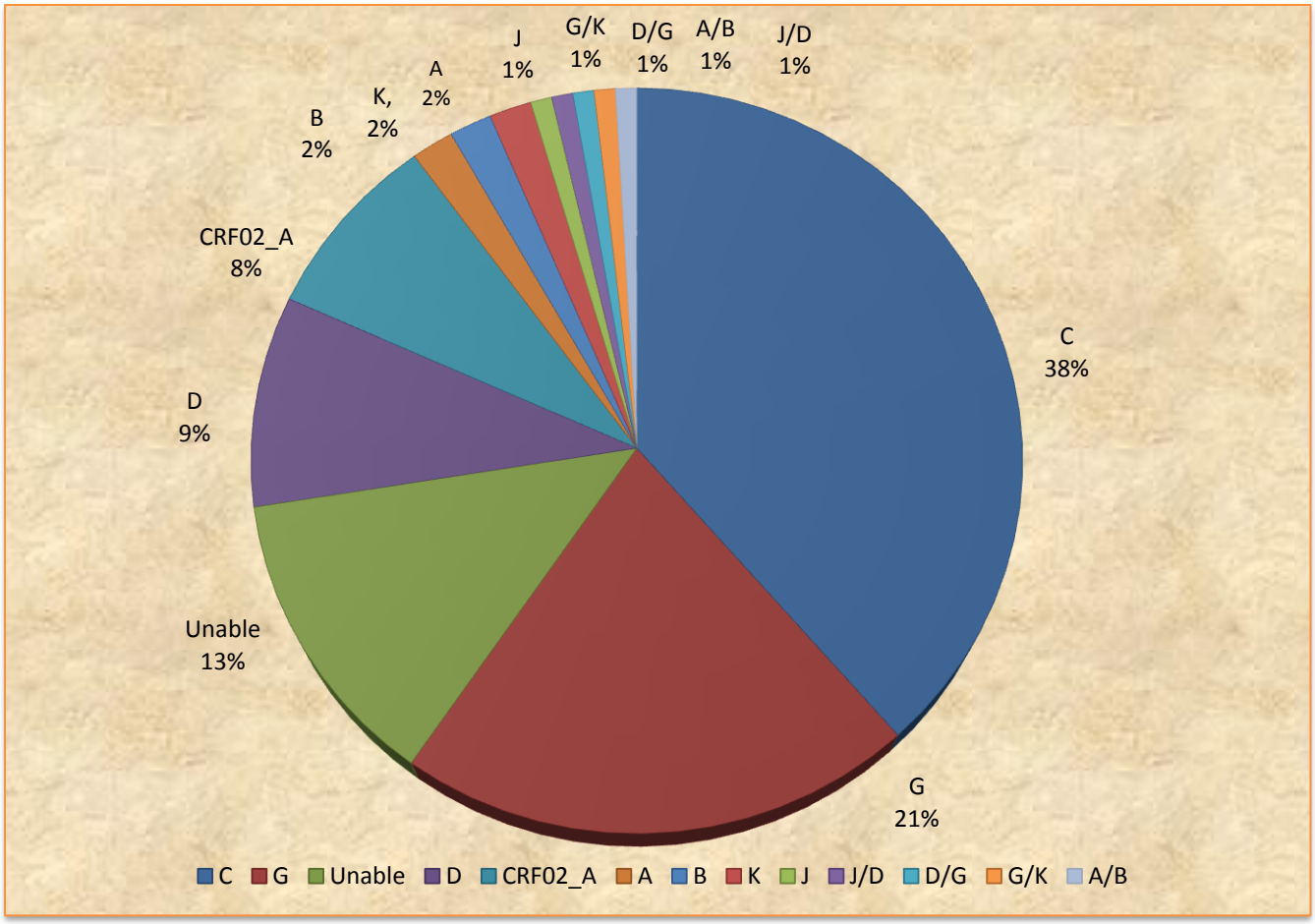


Figure (7-3) HIV-1 subtypes (NGS N=109)

There was no difference in prevalence of the most common subtypes C, G or D in patients diagnosed with HIV within the last 6 months, compared to those diagnosed for longer than 6 months (table 7-2)

		HIV duration	
HIV-1 Subtype	n/N	<6 months n/N	>6 months n/N
Unable	14/109	9/51	5/58
CRF02_A	8/95	5/42	3/53
C	42/95	18/42	23/53
G	23/95	10/42	13/53
A	2/95	1/42	1/53
D	10/95	4/42	6/53
K	2/95	0	2/53
J	1/95	1/42	0
B	2/95	0	2/53
J/D	1/95	1/42	0
D/G	1/95	0	0
G/K	1/95	0	1/53
A/B	1/95	1/42	0

Table (7-2) HIV subtypes (NGS method) and infection duration (N=95)

7.2. HIV-1 Subtypes and Resistance of ART classes

One patient 1/87 (1.1%) was noted to have significant mutations that confers resistance to NRTI. Most of the isolates were subtype C 41/109 (38%) and the mutations conferring resistance to NRTI was seen in 1/41 (2.4%) of these subtype C cases.

On the other hand we could not detect the subtype of 20/109 (18%) of the cases. Only 1/20 (5%) of these cases showed mutations conferring

resistance to NNRTI. 8/109 (7%) of the cases were CRF02_A subtype and 1/8 (12.5%) of these showed significant mutations that may lead to resistance to NNRTI. 4/41 (9.7%) of subtype C cases had mutations conferred resistance to NNRTI and 3/23 (13%) of subtype G showed mutations conferring resistance to NNRTI. 1/8 (12.5%) of CRF2_A subtype showed resistance to PI. Subtype C was the most common subtype with mutations to PI at 3/41 (7.3%) C. Only 1/23 (4.3%) of G subtypes cases showing a resistance mutation to PI. The proportion of resistant isolates in each clade is similar, although numbers are too small for statistical comparison.

				Resistance to NNRTI		**resistance to NRTI**		**Resistance to PI**	
		n/N	**%**	n/N	%	n/N	%	Count	%
HIV-1 Subtype	Unable	20/109	18%	1/20	5 %	0	0.0%	0	0%
	CRF02_A	8/89	9%	1/8	12.5%	0	0.0%	1/8	12.5%
	C	41/89	46%	4/41	9.7%	1/41	2.4%	3/41	7.3%
	G	23/89	26%	3/23	13%	0	0.0%	1/23	4.3%
	A	2/89	2.2%	0	0.0%	0	0.0%	0	0.0%
	D	10/89	11%	0	0%	0	0.0%	0	0%
	K	2/89	2.2%	0	0.0%	0	0.0%	0	0%
	J	1/89	1.2%	0	0.0%	0	0.0%	0	0%
	B	2/89	2.2%	0	0.0%	0	0.0%	1/2	50%
	total	109		9/87	10.3%	1/87	1.1%	6/93	6.5%

Table (7-3) HIV-1 Subtypes and resistance to ART classes (Sanger, N=89)

				Resistance to NNRTI		resistance to NRTI		Resistance to PI	
		n/N	%	n/N	%	n/N	%	Count	%
HIV-1 Subtype	Unable	14/109	12.8%	1/14	7.14%	0	0.0%	1/14	7.14%
	CRF02_A	8/95	8.4%	1/8	12.5%	0	0.0%	5/8	62.5%
	C	42/95	44.2%	6/42	14.3%	3/42	7.1%	5/42	11.9%
	G	23/95	24.2%	8/23	34.8%	0	0.0%	18/23	78.2%
	A	2/95	2.1%	0	0.0%	0	0.0%	0	0.0%
	D	10/95	10.5%	4/10	40%	1/10	10%	1/10	10%
	K	2/95	2.1%	1/2	50%	2/2	100%	1/2	50%
	J	1/95	1.1 %	0	0.0%	0	0.0%	1/1	100%
	B	2/95	2.1%	0	0.0%	0	0.0%	1/2	50%
	J/D	1/95	1.1 %	0	0.0%	0	0.0%	0	0.0%
	D/G	1/95	1.1 %	1/1	100%	0	0.0%	0	0.0%
	G/K	1/95	1.1 %	0	0.0%	0	0.0%	1/1	100
	A/B	1/95	1.1 %	0	0.0%	0	0.0%	0	0.0%
	total	109		24/105	22.9%	6/105	5.7%	34/105	32.4%

Table (7-4) HIV-1 Subtypes and resistance to ART classes (NGS, N=95)

7.3 Discussion

Detecting the HIV subtype is important for epidemiology and determining the infection origin. In this study subtype C was the most common (38%) followed by subtype G (21%). CRF02_A was found in 8% of the cases.

Previous phylogenetic analysis in 62 HIV positive Saudi patients, showed diversity of HIV subtypes in Saudi Arabia. The most common HIV-1 subtypes in the 2007 study were C (39.3%) G (25%), B (17.9%), D (3.6%), A (1.8%) and CRF02_AG (1.8%) (Badreddine et al. 2007).

In 2008 Al-Zahrani showed that HIV subtype C was the most common subtype in the Kingdom (58%). Other subtypes were B (17%) and A, D and G (8%) each. This study was performed in 39 HIV positive patients in Dammam, KSA (Alzahrani 2008).

In 2010 Jamjom et al studied 60 samples from HIV patients at King Abdulaziz University Hospital in Jeddah. The study was carried out using blood samples from treatment failure patients. The most frequent HIV-1 genotypes were type C (35%), G (38%), while type B (14%). All these studies favour an African or Asian source for most infections in Saudi patients. Genotype C of HIV-1 is the most frequent genotype in Africa and India, while genotype B is more prevalent in North America and Western Europe (Jamjoom et al. 2010).

In this study the most common HIV-1 group M subtype by using NGS method was subtype C (38%). Subtype G accounted for (21%) and subtype D for (9%). In addition, subtypes B (2%), A (2%), and CRF02_AG (8%) were present, and the subtype could not be identified in 13% of the cases. Other diversity of mixed recombinant strains consisted of CRF subtypes A/B, G/K, D/G, J/D (1% each). Considering the low prevalence of HIV in the Kingdom (0.1%) the diversity of HIV-1 phylogenetic subtypes is quite high.

The high diversity of subtypes suggests that the sources of HIV infection in the Kingdom are variable and indicates multiple sources.

These results were similar to previous HIV subtype studies in the Kingdom, but different from other studies in the Region (Table 7-5).

As the most common HIV-1 subtype in Saudi Arabia, identifying the geographical origin of the subtype C infections is important. HIV-1 subtype C is the most common worldwide and is the predominant strain circulating in India, South Africa, and countries along the east coast of Africa (Hu, Pieniazek & Mastro 2013).

The geographical proximity of Saudi Arabia to Africa and the common commercial interests explain the predominance of subtype C in the Kingdom. Subtype G has been found in the west central African countries of Nigeria, Cameroon, and Democratic Republic of Congo and patients with subtype G accounted for 21% of isolates in Jeddah. Again, CRF02_AG is the predominant HIV-1 strain circulating in west and west central Africa (Hu, Pieniazek & Mastro 2013) and accounted for 8% of our isolates.

Only 2% of cases in Jeddah had subtype B, which commonly circulates in the Americas and Europe including in MSM. In the study cohort (chapter 3) more than 10% of the cases had a past history of travelling abroad. This may also contribute to this diversity of HIV-1 phylogenetic subtypes.

A study in the neighbouring Yemen, showed that subtype B was the most common (47.3%) followed by subtype C (31.6%) and D (10.5%)(Saad et al. 2005). Moving round the Arabian region anticlockwise, there is a further shift in predominant subtype in Oman where the predominant subtype is B (56%) and A (12.5%)(Al Dhahry et al. 2004).

In Oman, other subtypes C, D and CRF01_AE were 8% each (Al Dhahry et al. 2004). This is surprising given the close historical and trading links with East Africa and one would expect there to be a larger proportion of clade C isolates. In this study 37% of HIV patients were treatment experience and 54% were treatment Naïve HIV patients. It is not known if this study results would be generalised to all of Oman, as it was done in Muscat.

Country	Selection ways		Author	Year	Subtype					
					A	B	C	G	D	CRFs
Iran	N patients	50 (51% IDU) ART naïve	Jahanbakhsh et al.	2013	-	-	-	-	-	CRF35_AD (95.7%) CRF01_AE (2.1%)
Lebanon	N patients	26 On ART	Pieniazek et al.	1998	44%	40%	4%	4%	4%	-
Oman	N patients	88 37 on ART 51 ART naïve	Al Dhahry et al.	2004	12%	56%	8%	-	8%	CRF01_AE (8%)
Saudi Arabia	N patients	62 On ART	Badreddine et al.	2007	1.8%	17.9%	39%	25%	3.6%	CRF02_AG(1.8 %)
	N patients	39 On ART	Alzahrani	2008	8%	17%	58%	8%	8%	
	N patients	60 On ART	Jamjoom et al.	2010	-	14%	35%	38%	%	-
	N patients	109 ART naïve	This study	2014	2%	2%	38%	21%	9%	CRF02_A (8%)
Turkey	N patients	117 ART naïve	Sayan et al.	2013	-	33%	-	-	-	CRF 02_AG, CRF 01_AE, CRF 12_BF and CRF 03_AB; 47%, 55/117
	N patients	190 ART naïve	Araştırılması et al.	2014	-	31%	-	-	-	CRF 02_AG, CRF 01_AE, CRF 12_BF, CRF 03_AB; (47%) CRF02_G (7.8%)
Yemen	N patients	19 On ART	Saad et al.	2005	5.3%	47.3%	31%		10.5%	

Table (7-5) HIV-1 Subtypes in Iran, Lebanon, Oman, Saudi Arabia, Turkey and Yemen

There is considerable genetic HIV variation in other countries in the Region. Phylogenetic relationships and transmission dynamics were analysed in 26 HIV-infected patients from Lebanon. One isolate was HIV-2 subtype B and the 25 HIV-1 isolates subtype were A (44%), B (40%), C (4%), D (4%) and G (4%)(Pieniazek et al. 1998). The predominance of subtype A and B suggest that most infection had been imported from Europe or North America, but the number of patients was very small and dates back nearly 2 decades.

In two reported Turkish studies, HIV-1 subtypes and CRFs were identified and the most common occurring subtypes were CRFs (CRF 02_AG, CRF 01_AE, CRF 12_BF and CRF 03_AB; 47%, 55/117) and B (33.3%, 39/117) (Sayan et al. 2013). A second study also showed that the most prevalent HIV-1 subtypes were subtype B (31%); recombinant B/CRF02_AG (10.5%) and CRF02_G (7.8%) (Araştırılması et al 2014). The studies were done in similar groups of ART naïve HIV positive patients from different Turkish cities. These different distributions of subtypes suggest a mixed importation of isolates from Europe and North America (subtype B) and other CRFs from Asia.

CRFs are more prominent in Iran where almost all strains were CRF35_AD, the study included 50 newly diagnosed with HIV infection from different Iranian centres, more than half of the patients were injecting drug users and needle sharing was common (Jahanbakhsh et al. 2013)

Unfortunately in this study permission was not granted to collect data on risk factors for acquiring HIV in Jeddah. As the regional variations in Table 7-5 show, it would be valuable to study this in more detail across the Kingdom to identify the probable sources of HIV infection imported by different risk groups in several cities.

The limited number of samples in the current study did not allow statistically significant comparison of ARV resistance rate in different HIV subtypes (Tables 7-3 and 7-4)

It is uncertain whether some subtypes are associated with resistance, and this deserves further study.

Chapter 8

Conclusions and recommendation

8. Conclusions and recommendations:

This study provides a more accurate picture of a large group of HIV patients attending a non-selective HIV clinic in Jeddah. The clinical and risk details are more complete compared to previous epidemiological reports from the Kingdom because details were obtained directly from patient notes. Details about coinfection were included as well as about clinical presentation and WHO staging details. Results differ from a very recent report from a tertiary reference centre in Riyadh. These are the only two such reports in the Arabian Peninsula.

However, data were not available consistently for all patients and this could be improved by regular quality checks and audits on operational performance in the clinic.

The sensitivity of HIV/AIDS subject in Saudi culture was one of the important difficulties we had to overcome with proper collaborations and close coordination with the Saudi authority and Ministry of Health in the Kingdom. Homosexuality (MSM) is considered as criminal action in Saudi Arabian law and if proved in court, sentence of death is the plenty. This made data collection about such very sensitive issue very

difficult and extra caution and absolute confidentiality is a must. It was one of the difficulties we had to tackle in this study.

8.1 Clinic Limitations

Data were collected retrospectively from patients' records, therefore, some key and important data could not be found (missing data). With retrospective studies, there is no control of accuracy on the available data because it depends on the quality of record keeping in the past. This was a particularly important limitation because some of the missing data were impossible to locate. For this study, all missing data were clearly labelled and excluded from all statistical calculations.

Hepatitis A (HAV) was not tested or it was not recorded in patients' medical notes at all in this study cohort. Moreover HAV vaccination was not recorded.

In addition, hepatitis B surface antigen (HBsAg) was not tested (missing) in 19% (262/1383) of the study cohort. HBsAg positive patients require further evaluation (HBV DNA) but this was not available in the records. There was no anti-HBV antibody testing so exposure to HBV could not be investigated. Data on HBV vaccination was not available

Hepatitis C (HCV IgG) was not tested (missing) in 20% (282/1383). Negative anti-HCV antibody results may be false negative in HIV

patients and HCV PCR is indicated both in this situation and in anti-HCV Ab positive cases. No HCV PCR results were available.

Hepatitis C coinfection may be missed if molecular methods are not used and hepatitis C coinfection needs early treatment. This should be reviewed prospectively in this cohort.

The recording of past infections e.g. toxoplasmosis and hepatitis B and VDRL results was highly variable and often deficient.

	test	n/N	%
Hepatitis A	anti HAV IgG	1383	100%
Hepatitis B	HBsAg	321/1383	23.2%
	HBV PCR	1383	100%
	anti HBV antibodies	1383	100%
Hepatitis C	anti HCV antibodies	282/1383	20%
	HCV PCR	1383	100%
Toxoplasma	IgG antibody	699/1383	50.5%
CMV	IgG antibody	486/1383	35%
EBV	IgG antibody	595/1383	43%
VZV	IgG antibody	767/1383	55.5%
Syphilis	VDRL	553/1383	40%

Table 8-1 Summary of missing results in 1383 HIV positive patients in Jeddah

The clinic still uses out-moded VDRL tests to look for syphilis. These tests are non- specific and may be altered by past bejel exposure. STI testing and screening protocols including introducing of modern syphilis serological methods need to be introduced as routine.

There is no clear protocol for detection or prevention of TB coinfection although this was common. This needs to be changed.

This study has provided very important data for public health services in the Kingdom and should help to improve the future HIV/AIDS plans in the country. This will reflect on the general Saudi population and particularly on HIV patients. Moreover, care of HIV patients will be improved to match (as much as possible) the best possible services in other countries.

Overall the study showed that clinical presentations and the rates of hepatitis and TB co infection are similar to those reported in many Western HIV patient cohorts. A substantial proportion of men acquire infection through sex with other men and this proportion is much higher than past reports from the Kingdom and Region. More patients were injecting drugs users than in previous reports from the Kingdom. This might be expected in Jeddah, where IDU has long been acknowledged to be present but is a concern in light of recent findings of increasing rates of HIV in IDU in Riyadh (Al-Shomrani 2014)

Coinfection rates are highest in difficult to reach groups such as drug users and those who have been in prison. This has difficult implications for the HIV control and treatment programmes in Jeddah and elsewhere in KSA.

HIV control strategies in the Middle East countries have challenges and difficulties. In Saudi Arabia, as in many other countries in the Region many social and public health issues should be considered. Although there has been a significant improvement in case notification of HIV/AIDS cases in the Kingdom, there are still serious challenges including social challenges facing the Saudi National Program for HIV Control. However, the Saudi national HIV prevention programme is one of the most comprehensive and transparent HIV control programmes in the Region, and needs to use data from studies such as these studies in the futures to respond to changes in this rising medical problem.

The best prevention approach is to improve the public health education and increase public awareness of HIV/AIDS. Political, financial, and social barriers have often restricted the most effective prevention and treatment strategies. These studies should also focus on detection and prevention in the high risk groups or difficult to reach groups such as IDU, MSM and prisoners. There is a need to ensure continuous access for high risk groups in order to ensure availability of treatment and all related services to control and prevent HIV/AIDS in such groups. Careful collaboration in the community between religious and social groups is very important to overcome these barriers.

For high risk groups including prisoners, HIV education should be provided.

HIV testing is indicated more frequently for those at increased risk of acquiring HIV (MSM, IDU, STI and Hepatitis/TB clinics). HIV testing should be encouraged at all clinics whenever HIV is suspected.

HIV patients should be screened more vigorously and systematically for possible coinfections and opportunistic infections (OI) using protocols adapted from other international standards eg BHIVA, CDC. Clinics in the Kingdom should have standard proformas for clinical data collection and guidelines for CD4 and viral load monitoring. For hepatitis screening we recommend that HAV to be included.

This study did not examine the success of ART regimen, but this should be audited routinely in future. Use of correct HIV treatment protocols for coinfected patients needs to be checked by clinical audit.

8.2 Antiretroviral resistance

Clinically significant ART resistance is emerging (16%) in this population. A variety of other markers included some clustering suggest local transmission of primary resistance. The results are probably generalizable in KSA and we recommend the introduction of routine resistance testing for all HIV positive patients in resource rich countries in the Region before starting ART. The WHO guidelines for HIV/AIDS which includes Saudi Arabia within

the MENA region should be revised, considering that the Kingdom and other GCC are resource rich. Local and national management protocols should include the best HIV/AIDS management standards rather than following recommendations for resource poor countries in Africa.

The deep sequencing method was more accurate and a sensitive and has high reproducibility of test results compared to the original (standard) sequencing technique. It is suggested that this should be available for use in selected cases and specific prospective population studies are needed to provide more accuracy in identifying pattern of ART resistance in the Kingdom.

Regimens of ART should be selected based on resistance test results with consideration of dosing frequency, pill burden, toxicity profiles, comorbidities, and drug-drug interactions for each patient individually.

As future work we recommend that similar studies be carried out simultaneously in all Saudi provinces, and that collaborations with international AIDS organizations be developed in order to improve epidemiological and clinical data in the Kingdom.

Moreover, future studies of a larger cohort from Saudi Arabia including ART naïve HIV patients are indicated to reveal the changing real state of the transmitted drug resistance in the country. This is the only study of treatment naïve patients in the Kingdom.

For this study we only collected limited epidemiological data and were not able to look for clustering of resistance in groups of patients. Further studies about epidemiological risk for primary and drug induced ART resistance are needed to help predict future ART resistance in HIV patients in the Kingdom, and to improve or adapt treatment protocols.

HIV phylogenicity and subtyping in Saudi Arabia showed high diversities in four studies (including ours) in Saudi Arabia. These data about HIV-1 genetic origin and possible HIV geographical sources of infection help to inform for future plans and for control of HIV/AIDS in the Kingdom. As a future work we recommend wider scale studies and more research projects to explore HIV strain diversity in Saudi Arabia, and use the data to focus prevention effects on specific high risk area or newly emerging foci of transmission.

8.3 Conclusion

This work was carried out within the social and administrative climate several years ago, and I thank all those who contributed and helped to overcome a number of obstacles. The work described in this thesis has successfully addressed the aims at the outset

- It has provided a clear picture of clinical presentations of a large cohort of patients in Jeddah.

- It has provided a more complete picture of risk factors for infection including the hard to reach groups such as MSM, IDU and prisoners, and confirmed the expected links of these groups to coinfections such as HBV, HCV, TB and syphilis.
- It has revealed a lack of consistency of approach to screening and recording opportunistic infections and interventions to prevent these.
- In the face of lack of the specific management protocols it could not assess the success of therapy in this cohort.
- The study is the first comprehensive and study of transmitted drug resistance in unselected ART- naïve patients in the Region and the first to compare results of Sanger genoptypic sequencing with next generation sequencing.
- These molecular methods revealed rates of primary resistance of 5-15% against several classes of antiretroviral drugs, including etravirine and rilpivirne, while have hardly been used in the Kingdom until now.
- The results suggest that all patients should have resistance testing as a routine prior to starting therapy and whenever there is treatment failure.
- The study confirms the broad pattern of heterogeneity of HIV isolates in KSA described in previous studies. These are different from the countries in the Region, highlighting their

use in identifying risk groups for importation of HIV infection into the Kingdom.

The Ministry of Health and other officials have established an innovative programme for HIV in the Kingdom, against a backdrop of social complexities. Standards of care are already well established and have been upgraded since the first part of this work was completed. The work described in this thesis suggests that the clinic and management protocols should be reviewed regularly in comparison to other international standards of care and protocols in resource rich countries, and adapted to match these. Regular clinical and operational audit of care holding, treatment initiation and success and similar outcomes, should be instituted to monitor the effectiveness of the programme.

These efforts will cause benefit not only for patients who currently live with HIV/AIDS in the Kingdom, but also reduce the risks of onward transmission of potentially resistant HIV within Saudi Arabia, and benefit the country as a whole.

References:

Abaalkhail, F., Elsiesy, H., AlOmair, A., Alghamdi, M.Y., Alalwan, A., AlMasri, N. & Al-Hamoudi, W. (2014) 'SASLT practice guidelines for the management of hepatitis B virus', *Saudi Journal of Gastroenterology*, vol. 20, no. 1, p. 5-6.

Abdo, A.A., Sanai, F.M. & Al-Faleh, F.Z. (2012) 'Epidemiology of viral hepatitis in Saudi Arabia: are we off the hook?', *Saudi Journal of Gastroenterology*, vol. 18, no. 6, p. 349-357.

Abo, Y., Djimon, M.Z., Messou, E., Balestre, E., Kouakou, M., Akakpo, J., Ahouada, C., de Rekeneire, N., Dabis, F. & Lewden, C. (2015) 'Severe morbidity after antiretroviral (ART) initiation: active surveillance in HIV care programs, the IeDEA West Africa collaboration', *BMC Infectious Diseases*, vol. 15, no. 1, p. 176.

Abu-Raddad, L.J. & Longini, I.M., Jr. (2008) 'No HIV stage is dominant in driving the HIV epidemic in sub-Saharan Africa', *AIDS*, vol. 22, no. 9, pp. 1055-1061.

Agacfidan, A., Kaiser, R. & Akgul, B. (2014) 'HIV in Turkey, a country bridging the Islamic world and Europe', *Journal of Infection and Public Health*, vol. 7, no. 3, pp. 249-250.

Akala, F.A. & Semini, I. (2010) *Characterizing the HIV/AIDS epidemic in the Middle East and North Africa: time for strategic action*, World Bank Publications.WHO, Geneva.

Akbar, H.O. (2004) 'Hepatitis C virus infection in Saudi Arabia', *Saudi Journal of Gastroenterology*, vol. 10, no. 3, pp. 127-131.

Akhtar, S. & Mohammad, H.G.H.H. (2012) 'Time series cross-correlation analysis of HIV seropositivity and pulmonary tuberculosis among migrants entering Kuwait', *International Journal of Mycobacteriology*, vol. 1, no. 1, pp. 29-33.

Al-Dabal, L., Badreddin, S., Abro, A. & Javeed, N.Y. (2011) 'Prevalence and pattern of pulmonary infections among HIV/AIDS adults patients admitted to a tertiary care hospital, Dubai, United Arab Emirates, 2009-2010', *European Respiratory Journal*, vol. 38, Suppl 55, p. 25-29.

Al-Dhahry, S.H., Scrimgeour, E.M., Al Suwaid, A.R., Al Lawati, M.R., El Khatim, H.S., Al Kobaisi, M.F. & Merigan, T.C. (2004) 'Human

immunodeficiency virus type 1 infection in Oman: antiretroviral therapy and frequencies of drug resistance mutations', *AIDS Research Human Retroviruses*, vol. 20, no. 11, pp. 1166-1172.

Al Hajjar, S.H., Frayha, H. & Althawadi, S. (2011) 'Antiretroviral resistance in HIV-infected Saudi children failing first-line highly active antiretroviral therapy', *Annals of Saudi Medicine*, vol. 32, no. 6, pp. 565-569.

Al-Haddad, M., Baig, B. & Ebrahim, R. (1997) 'Epidemiology of HIV and AIDS in Bahrain', *Journal of Communicable Diseases*, vol. 29, no. 4, pp. 321-328.

Al-Haddad, M., Khashaba, A., Baig, B. & Khalfan, S. (1994) 'HIV antibodies among intravenous drug users in Bahrain', *Journal of Communicable Diseases*, vol. 26, no. 3, pp. 127-132.

Al-Hajoj, S. & Varghese, B. (2015) 'Tuberculosis in Saudi Arabia: the journey across time', *Journal of Infection in Developing Countries*, vol. 9, no. 03, pp. 222-231.

Al-Hajoj, S.A. (2009) 'Can we change the way we look at BCG vaccine?', *Annals of Thoracic Medicine*, vol. 4, no. 2, pp. 92-93; author reply 93-94.

Alhuraiji, A., Alaraj, A., Alghamdi, S., Alrbiaan, A., & Alrajhi, A.A. (2014) 'Viral hepatitis B and C in HIV-infected patients in Saudi Arabia', *Annals of Saudi Medicine*, vol. 34, no. 3, pp. 207-210

Al-Jabri, A.A., Al-Muharrami, Z.K., Balkhair, A.A. & Ganguly, S.S. (2010) 'The importance of HIV antenatal screening programs for pregnant women', *Saudi Medical Journal*, vol. 31, no. 1, pp. 64-68.

Al-Mazrou, Y.Y., Al-Jeffri, M.H., Fidail, A.I., Al-Huzaim, N. & El-Gizouli, S.E. (2005) 'HIV/AIDS epidemic features and trends in Saudi Arabia', *Annals of Saudi Medicine,* vol. 25, no. 2, pp. 100-104.

AL-Malki, B.M. (2014) 'Knowledge and awareness of sexually transmitted disease among male university students in Taif, Saudi Arabia', *International Journal of Medical Science and Public Health*, vol. 3, no. 3, pp. 342-348.

Al-Mozaini, M., Mk, M., Al-Hokail, A., Mohmed, M. & Daham, M. (2014) 'HIV-Care Outcome in Saudi Arabia; a longitudinal cohort', J*ournal of AIDS and Clinical Research*, vol. 5, no. 370, p. 2.

Alim, A., Artan, M.O., Baykan, Z. & Alim, B.A. (2009) 'Seroprevalence of hepatitis B and C viruses, HIV, and syphilis infections among engaged couples', *Saudi Medical Journal*, vol. 30, no. 4, pp. 541-545.

Alothman, A., Altalhi, K., Saedy, A.A. & Enazi, T.A. (2010) 'What is the real prevalence of HIV-infection in Saudi Arabia?', *Infectious Diseases: Research and Treatment*, vol. 3, no. 2215, p. 41.

Alrajhi, A.A. (2004) 'Human immunodeficiency virus in Saudi Arabia', *Saudi Medical Journal*, vol. 25, no. 11, pp. 1559-1563.

Alshomrani, A. T. (2014). HIV, HCV, HBV prevalence among heroin addicts in Saudi Arabia. *Drug & Alcohol Dependence*, vol. 3, pp *140*.

Alzahrani, A.J., Obeid, O.E., Al-Ali, A. & Imamwardi, B. (2009) 'Detection of hepatitis C virus and Human immunodeficiency virus in expatriates in Saudi Arabia by antigen-antibody combination assays', *Journal of Infection in Developing Countries*, vol. 3, no. 3, pp. 235-238.

Alzahrani, A.J. (2008) 'Analysis of HIV subtypes and the phylogenetic tree in HIV-positive samples from Saudi Arabia', *Saudi Medical Journal*, vol. 29, no. 10, pp. 1394-1396.

Andre, F. (2000) 'Hepatitis B epidemiology in Asia, the Middle East and Africa', *Vaccine Journal*, vol. 18 Suppl 1, pp. S20-22.

Antonucci, G., Girardi, E., Raviglione, M.C. & Ippolito, G. (1995) 'Risk factors for tuberculosis in HIV-infected persons. A prospective cohort study. The Gruppo Italiano di Studio Tubercolosi e AIDS (GISTA)'*, Journal od American Medical Association*, vol. 274, no. 2, pp. 143-148.

Araştırılması, P.İ.D.M., Yalcinkaya, T. & Köse, Ş. (2014) 'Özgün Çalışma/Original Article Mikrobiyol Bul 2014; 48 (4): 585-595', *Mikrobiyol Bul*, vol. 48, no. 4, pp. 585-595.

Asghar, R.J. (2014) 'Hepatitis A and E: not to be forgotten', *Eastern Mediterranean Health Journal*, vol. 20, no. 3, pp. 212-213.

Asahchop, E.L., Wainberg, M.A., Oliveira, M., Xu, H., Brenner, B.G., Moisi, D., Ibanescu, I.R. & Tremblay, C. (2013) 'Distinct resistance patterns to etravirine and rilpivirine in viruses containing nonnucleoside reverse transcriptase inhibitor mutations at baseline', *AIDS,* vol. 27, no. 6, pp. 879-887.

Aylikçi, B.U., Bamise, C.T., Hamidi, M.M., Turkal, M. & Çolak, H. (2013) 'Human immunodeficiency virus/acquired immunodeficiency syndrome knowledge among high school students in Kirikkale province of Turkey', *Journal of Natural Science, Biology and Medicine*, vol. 4, no. 1, pp. 81-86.

Badahdah, A.M. (2010) 'Stigmatization of persons with HIV/AIDS in Saudi Arabia', *Journal of Transcultural Nursing*, vol. 21, no. 4, pp. 386-392.

Badreddine, S., Smith, K., Van Zyl, H., Bodelle, P., Yamaguchi, J., Swanson, P., Devare, S.G. & Brennan, C.A. (2007) 'Identification and characterization of HIV type 1 subtypes present in the Kingdom of Saudi Arabia: high level of genetic diversity found', *AIDS research and human retroviruses*, vol. 23, no. 5, pp. 667-674.

Baesi, K., Ravanshad, M., Ghanbarisafari, M., Saberfar, E., SeyedAlinaghi, S. & Volk, J.E. (2014) 'Antiretroviral drug resistance among antiretroviral-naïve and treatment experienced patients infected with HIV in Iran', *Journal of medical virology*, vol. 86, no. 7, pp. 1093-1098.

Bakri, F. G., AL-Azzeh, R. S., Irshaid, A. A. & Hijjawi, B. (2010) 'Human immunodeficiency virus disease in Jordan—data from the National AIDS Program from 1986 until 2008', *International Journal of Infectious Diseases,* vol. 14**,** pp. e923-e924.

Ball, A.L., Rana, S. & Dehne, K.L. (1998) 'HIV prevention among injecting drug users: responses in developing and transitional countries', *Public Health Report*s, vol. 113, no. Suppl 1, p. 170.

Balkhair, A.A., Al-Muharrmi, Z.K., Ganguly, S. & Al-Jabri, A.A. (2012) 'Spectrum of AIDS defining opportunistic infections in a series of 77 hospitalised HIV-infected Omani patients', *Sultan Qaboos University Medical Journal*, vol. 12, no. 4, pp. 442-448.

Bangsberg, D.R., Moss, A.R. & Deeks, S.G. (2004) 'Paradoxes of adherence and drug resistance to HIV antiretroviral therapy', *Journal of Antimicrobial Chemotherapy*, vol. 53, no. 5, pp. 696-699.

Bawah, A., Bongaarts, J., Greenhalgh, S., McNicoll, G. & Montgomery, M. (2006) 'AIDS, epidemic update: December 2005', *Population and Development Review*, vol. 32, no. 1, pp. 184-184.

Bawazir, A.A., Hart, C.A., Sallam, T.A., Parry, C.M., Beeching, N.J. & Cuevas, L.E. (2010) 'Seroepidemiology of hepatitis A and hepatitis E viruses in Aden, Yemen', *Transactions of the Royal Society of Tropical Medicine and Hygiene*, vol. 104, no. 12, pp. 801-805.

British HIV Association. BHIVA, (2007) Guidelines on antiretroviral therapy and testing [Online] available from: http://www.bhiva.org/documents/Guidelines/Standards/StandardsHIVClinicalCare.pdf [Accessed 20.05.2015 16:00]

Berger, S. (2015) Infectious Diseases of Saudi Arabia, GIDEON Informatics Inc. PP. 159-160.

British HIV Association. BHIVA, (2013) Standards of Care for People Living with HIV 2013 [Online] avsilable from:

http://www.bhiva.org/documents/Standards-of-care/BHIVAStandardsA4.pdf[Accessed 20.05.2015 20:00]

Broekmans, J.F., Migliori, G.B., Rieder, H.L., Lees, J., Ruutu, P., Loddenkemper, R. & Raviglione, M.C. (2002) 'European framework for tuberculosis control and elimination in countries with a low incidence. Recommendations of the World Health Organization (WHO), International Union Against Tuberculosis and Lung Disease (IUATLD) and Royal Netherlands Tuberculosis Association (KNCV) Working Group', *European Respiratory Journal*, vol. 19, no. 4, pp. 765-775.

Brown, T., Bao, L., Raftery, A.E., Salomon, J.A., Baggaley, R.F., Stover, J. & Gerland, P. (2010) 'Modelling HIV epidemics in the antiretroviral era: the UNAIDS Estimation and Projection package 2009', *Sexually Transmitted Infections*, vol. 86 Suppl 2, pp. ii3-10.

Bruchfeld, J., Correia-Neves, M. & Källenius, G. (2015) 'Tuberculosis and HIV Coinfection', *Cold Spring Harbor Perspectives in Medicine*, p. 17871.

Booth C. Transmitted resistance. Chapter 10 In: Geretti AM, editor. *Antiretroviral Resistance in Clinical Practice*. London: Mediscript; 2006.. Available from: http://www.ncbi.nlm.nih.gov/books/NBK2242/(Accessed 5 April 2015 15:00).

Bozicevic, I., Riedner, G. & Haghdoost, A. (2014) 'HIV case reporting in the countries of North Africa and the Middle East', *Journal of the International AIDS Society,* vol. 17, no. 1.

Burnett, R.J., Francois, G., Kew, M.C., Leroux-Roels, G., Meheus, A., Hoosen, A.A. & Mphahlele, M.J. (2005) 'Hepatitis B virus and human immunodeficiency virus co-infection in sub-Saharan Africa: a call for further investigation', *Liver International*, vol. 25, no. 2, pp. 201-213.

Centers for Disease Control and Prevention (U.S.) (1981) *Reports on AIDS,* Dept. of Health and Human Services, Public Health Service, Centers for Disease Control, Atlanta, GA. Available from http://www.bt.cdc.gov/agent/smallpox/response-plan/files/guide-d.pdf (Accessed 5 June 2012).

Centers for Disease Control and Prevention (U.S.) (2010). *Specimen Collection and Transport Guidelines* [Online]. Centers for Disease Control, Atlanta, GA. (Accessed 5 May 2015)

Centers for Disease Control and Prevention (U.S.) (2014) *'Preexposure prophylaxis for the prevention of HIV infection in the United States–2014: a clinical practice guideline*', Centers for disease control and prevention. (Accessed 2 Jun 2015)

Chang, C.C., Crane, M., Jaworowski, A., Lloyd, A., Martyn, A. & Lewin, S.R. (2014) Immunopathogenesis of HIV Coinfections, in, *Encyclopedia of AIDS*, Springer, pp. 1-15.

Chasela, C.S., Kourtis, A.P., Wall, P., Drobeniuc, J., King, C.C., Thai, H., Teshale, E.H., Hosseinipour, M., Ellington, S., Codd, M.B., Jamieson, D.J., Knight, R., Fitzpatrick, P., Kamili, S., Hoffman, I., Kayira, D., Mumba, N., Kamwendo, D.D., Martinson, F., Powderly, W., Teo, C.-G. & van der Horst, C. (2014) 'Hepatitis B virus infection among HIV-infected pregnant women in Malawi and transmission to infants', *Journal of Hepatology*, vol. 60, no. 3, pp. 508-514

Chemtob, D. & Srour, S.F. (2005) 'Epidemiology of HIV infection among Israeli Arabs', *Public Health*, vol. 119, no. 2, pp. 138-143.

Chemtob, D. & Grossman, Z. (2004) 'Epidemiology of adult and adolescent HIV infection in Israel: a country of immigration', *International Journal of STD & AIDS*, vol. 15, no. 10, pp. 691-696.

Chimphambano, C., Komolafe, I. & Muula, A. (2007) 'Prevalence of HIV, HepBsAg and Hep C antibodies among inmates in Chichiri prison, Blantyre, Malawi', *Malawi Medical Journal,* vol. 19, no. 3, p. 107.

Country Progress Report Sultanate Oman (2014) *Global AIDS Response Progress Report* [Online] Available from: http://www.unaids.org/sites/default/files/country/documents//OMN_narrative_report_2014.pdf (Accessed: 20.05.2015).

Corbett, E.L., Watt, C.J., Walker, N., Maher, D., Williams, B.G., Raviglione, M.C. & Dye, C. (2003) 'The growing burden of tuberculosis: global trends and interactions with the HIV epidemic', *Archives of Internal Medicine*, vol. 163, no. 9, pp. 1009-1021.

Crum-Cianflone, N.F., Hullsiek, K.H., Roediger, M., Ganesan, A., Patel, S., Landrum, M.L., Weintrob, A., Agan, B.K., Medina, S. & Rahkola, J. (2010) 'A randomized clinical trial comparing revaccination with pneumococcal conjugate vaccine to polysaccharide vaccine among HIV-infected adults', *Journal of Infectious Diseases*, vol. 202, no. 7, pp. 1114-1125.

Davarpanah, M., Rafiee, G. & Mehrabani, D. (2009) 'The prevalence of M. tuberculosis infection and disease in HIV positive individuals in Shiraz, Southern Iran', *Iranian Red Crescent Medical Journal*, vol. 11, no. 2, p. 199.

Deshpande, A., Karki, S., Recordon-Pinson, P. & Fleury, H.J. (2011) 'Drug resistance mutations in HIV type 1 isolates from naïve patients eligible for first line antiretroviral therapy in JJ Hospital, Mumbai, India', *AIDS Research and Human Retroviruses*, vol. 27, no. 12, pp. 1345-1347.

Ehrahim, R.A., Farid, E.M., Yousif, A. & Jamsheer, A.E. (2002) 'Microbiological infections in HIV positive Bahraini patients with low CD4+ T-lymphocyte count', *Journal of Communcable Diseases,* vol. 34, no. 3, pp. 160-170.

El-Hazmi, M.M. (2004) ' Prevalence of HBV, HCV, HIV-1, 2 and HTLV-I/II infections among blood donors in a teaching hospital in the Central region of Saudi Arabia', *Saudi Medical Journal*, vol. 25, no. 1, pp 26-33.

El-Sadr, W.M. & Tsiouris, S.J. (2008) 'HIV-associated tuberculosis: diagnostic and treatment challenges', *Seminars in Respiratory* and *Critical Care Medicine* , vol. 29, no. 5, pp. 525-531.

El-Serag, H.B. (2012) 'Epidemiology of viral hepatitis and hepatocellular carcinoma', *Gastroenterology*, vol. 142, no. 6, pp. 1264-1273. e1261.

Ellis, M.E., Halim, M.A., Frayha, H., Bernvil, S., Sheth, K. & Alabduljabbar, J.O. (1993) 'HIV-infection in Saudi Arabia - occurrence, pattern of disease and future implications', *Saudi Medical Journal*, vol. 14, no. 4, pp. 325-333.

Enanoria, W.T., Ng, C., Saha, S.R. & Colford Jr., J.M.C. (2004) 'Treatment outcomes after highly active antiretroviral therapy: a meta-analysis of randomised controlled trials', *Lancet Infectious Diseases*, vol. 4, no. 7, pp. 414-425.

Erice, A., Mayers, D.L., Strike, D.G., Sannerud, K.J., McCutchan, F.E., Henry, K. & Balfour, H.H., Jr. (1993) 'Brief report: primary infection with zidovudine-resistant human immunodeficiency virus type 1', *New England Journal of Medicine*, vol. 328, no. 16, pp. 1163-1165.

Fallahzadeh, H., Morowatisharifabad, M. & Ehrampoosh, M.H. (2009) 'HIV/AIDS epidemic features and trends in Iran, 1986-2006', *AIDS Behaviour*, vol. 13, no. 2, pp. 297-302.

Fageeh, W.M. (2013) 'Sexually transmitted infections among patients with herpes simplex virus at King Abdulaziz University Hospital', *BioMedCentral Research Notes*, vol. 6, no. 1, p. 301.

Fageeh, W., Iyer, A., Almalki, N., Alturkistani, W. & Yaghmoor, S. (2014) 'Prevalence and awareness of sexually transmitted infections among inmates of a drug rehabilitation center in Saudi Arabia: a cross-sectional study', *Epidemiology*, vol. 4, no. 154, pp. 2161-65.

Fischl, M.A., Richman, D.D., Grieco, M.H., Gottlieb, M.S., Volberding, P.A., Laskin, O.L., Leedom, J.M., Groopman, J.E., Mildvan, D., Schooley, R.T., Jackson, G.G., Durack, D.T. & King, D. (1987) 'The efficacy of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-

Related Complex', *New England Journal of Medicine*, vol. 317, no. 4, pp. 185-191.

Flepp, M., Schiffer, V., Weber, R. & Hirschel, B. (2001) 'Modern anti-HIV therapy', *Swiss Medical Weekly*, vol. 131, no. 15-16, pp. 207-213.

Frentz, D., Boucher, C. & Van De Vijver, D. (2012) 'Temporal changes in the epidemiology of transmission of drug-resistant HIV-1 across the world', *AIDS Review,* vol. 14, no. 1, pp. 17-27.

Ford, N., Mayer, K.H., Barlow, L., Bagyinszky, F., Calmy, A., Chakroun, M., Casas, E., Dominguez, K., Kaplan, J. & Green, K. (2015) 'World Health Organization Guidelines on Postexposure Prophylaxis for HIV: Recommendations for a Public Health Approach', *Clinical Infectious Diseases,* vol. 60, no. suppl 3, pp. S161-S164.

Gebremaryam, T. (2014). *HIV/AIDS and pregnancy* [Online]. Available from http://www.slideshare.net/mesfinmulugeta524/hiv-pregnancy-42708612 (Accessed 13 January 2015).

Geretti, A.M. & Easterbrook, P. (2001) 'Antiretroviral resistance in clinical practice', *International Jurnal of STD & AIDS*, vol. 12, no. 3, pp. 145-153.

Geretti, A.M., Patel, M., Sarfo, F.S., Chadwick, D., Verheyen, J., Fraune, M., Garcia, A. & Phillips, R.O. (2010) 'Detection of highly prevalent hepatitis B virus coinfection among HIV-seropositive persons in Ghana', *Journal of Clinical Microbiology*, vol. 48, no. 9, pp. 3223-3230.

Geretti, A.M., Conibear, T., Hill, A., Johnson, J.A., Tambuyzer, L., Thys, K., Vingerhoets, J., Van Delft, Y., Rieger, A. & Vetter, N. (2014) 'Sensitive testing of plasma HIV-1 RNA and Sanger sequencing of cellular HIV-1 DNA for the detection of drug resistance prior to starting first-line antiretroviral therapy with etravirine or efavirenz', *Journal of Antimicrobial Chemotherapy*, vol. 69, no. 4, pp. 1090-1097.

Grant, R.M. & Liegler, T. (2015) 'Weighing the risk of drug resistance with the benefits of HIV preexposure prophylaxis', *Journal of Infectious Diseases*, p. 678.

Gray, P.B. (2004) 'HIV and Islam: is HIV prevalence lower among Muslims?', *Social Science and Medicine*, vol. 58, no. 9, pp. 1751-1756.

Group, D.C.O.A.E.O.A.-H.D.S. (2006) 'Liver-related deaths in persons infected with the human immunodeficiency virus: the D: A: D study', *Archives of Internal Medicine*, vol. 166, no. 15, p. 1632.

Group, E.S. (2014) 'Efficacy of 400 mg efavirenz versus standard 600 mg dose in HIV-infected, antiretroviral-naïve adults (ENCORE1): a randomised,

double-blind, placebo-controlled, non-inferiority trial', *Lancet*, vol. 383, no. 9927, pp. 1474-1482.

Golub, J.E., Cohn, S., Saraceni, V., Cavalcante, S.C., Pacheco, A.G., Moulton, L.H., Durovni, B. & Chaisson, R.E. (2015) 'Long-term Protection From Isoniazid Preventive Therapy for Tuberculosis in HIV-Infected Patients in a Medium-Burden Tuberculosis Setting: The TB/HIV in Rio (THRio) Study', *Clinical Infectious Diseases*, vol. 60, no. 4, pp. 639-645.

Hammer, S.M. (2005) 'Single-dose nevirapine and drug resistance: the more you look, the more you find', *Journal of Infectious Diseases*, vol. 192, no. 1, pp. 1-3.

Hasnain, M. (2005) 'Cultural approach to HIV/AIDS harm reduction in Muslim countries', *Harm Reduction Journal*, vol. 2, p. 23.

Hegazi, A., Ramskill, N., Norbrook, M., Dwyer, E., Milne, S., Nathan, B., Esterich, S., ElGalib, A., Morgan, T. & Barbour, A. (2015) Genital tract infections in HIV-infected pregnant women in South West London, *HIV Medicine*, vol. 16, pp. 11-11.

Hemelaar, J., Gouws, E., Ghys, P.D. & Osmanov, S. (2006) 'Global and regional distribution of HIV-1 genetic subtypes and recombinants in 2004', *AIDS*, vol. 20, no. 16, pp. W13-23.
Hirsch, M.S., Gunthard, H.F., Schapiro, J.M., Brun-Vezinet, F., Clotet, B., Hammer, S.M., Johnson, V.A., Kuritzkes, D.R., Mellors, J.W., Pillay, D., Yeni, P.G., Jacobsen, D.M. & Richman, D.D. (2008) 'Antiretroviral drug resistance testing in adult HIV-1 infection: 2008 recommendations of an International AIDS Society-USA panel', *Clinical Infectious Diseases*, vol. 47, no. 2, pp. 266-285.

HIV Web Study. (2007) *Antiretroviral Therapy Adherence in the Homeless* [Online] Available from http://depts.washington.edu/hivaids/spop/case5/index.shtml (Accessed 13 January 2015).

Hope, V., McVeigh, J., Marongiu, A., Evans-Brown, M., Smith, J., Kimergard, A., Croxford, S., Beynon, C., Parry, J. & Bellis, P. (2013) Prevalence of, and risk factors for, human immunodeficiency virus, hepatitis B and hepatitis C infections among men who inject image-and performance-enhancing drugs in England & Wales, *BMJ Open.* 2013 Sep 12;3(9):e003207.

Hu, D.J., Pieniazek, D. & Mastro, T.D. (2013) 'The genetic diversity and global molecular epidemiology of HIV', *AIDS and other manifestation of HIV infection*.

Hurst, S.A., Appelgren, K.E. & Kourtis, A.P. (2014) 'Prevention of mother-to-child transmission of HIV Type 1: the role of neonatal and infant

prophylaxis', *Expert Review of Anti-infective Therapy*, vol. 13, no. 2, pp. 169-181.

Huang, H.Y., Daar, E., Sax, P., Young, B., Cook, P., Benson, P., Cohen, C., Scribner, A. & Hu, H. (2008) 'The prevalence of transmitted antiretroviral drug resistance in treatment-naïve patients and factors influencing first-line treatment regimen selection', *HIV Medicine*, vol. 9, no. 5, pp. 285-293.

Ivers, L.C., Kendrick, D. & Doucette, K. (2005) 'Efficacy of antiretroviral therapy programs in resource-poor settings: a meta-analysis of the published literature', *Clinical Infectious Diseases*, vol. 41, no. 2, pp. 217-224.

Jahanbakhsh, F., Hattori, J., Matsuda, M., Ibe, S., Monavari, S.-H.R., Memarnejadian, A., Aghasadeghi, M.R., Mostafavi, E., Mohraz, M. & Jabbari, H. (2013) 'Prevalence of transmitted HIV drug resistance in Iran between 2010 and 2011', *PloS One*, vol. 8, no. 4, p. e61864.

Jamjoom, G.A., Azhar, E.I., Madani, T.A., Hindawi, S.I., Bakhsh, H.A. & Damanhouri, G.A. (2010) 'Genotype and antiretroviral drug resistance of human immunodeficiency virus-1 in Saudi Arabia', *Saudi Medical Journal*, vol. 31, no. 9, pp. 987-992.

Johnson, V.A., Brun-Vezinet, F., Clotet, B., Kuritzkes, D.R., Pillay, D., Schapiro, J.M. & Richman, D.D. (2006) 'Update of the drug resistance mutations in HIV-1: Fall 2006', *Topics in HIV Medicine*, vol. 14, no. 3, pp. 125-130.

Kabbash, I.A., Felemban, S.M., Stephens, G.M., Al-Hakeem, R.F., Zumla, A.I. & Memish, Z.A. (2012) 'HIV case notification rates in the Kingdom of Saudi Arabia over the past decade (2000–2009)', *PloS One*, vol. 7, no. 9, p. e45919.

Karageorgopoulos, D.E., El-Sherif, O., Bhagani, S. & Khoo, S.H. (2014) 'Drug interactions between antiretrovirals and new or emerging direct-acting antivirals in HIV/hepatitis C virus coinfection', *Current Opinion in Infectious Diseases*, vol. 27, no. 1, pp. 36-45.

Kermode, M. (2004) 'Unsafe injections in low-income country health settings: need for injection safety promotion to prevent the spread of blood-borne viruses', *Health Promotion International*, vol. 19, no. 1, pp. 95-103.

Konopnicki, D., Mocroft, A., De Wit, S., Antunes, F., Ledergerber, B., Katlama, C., Zilmer, K., Vella, S., Kirk, O. & Lundgren, J.D. (2005) 'Hepatitis B and HIV: prevalence, AIDS progression, response to highly active antiretroviral therapy and increased mortality in the EuroSIDA cohort', *AIDS*, vol. 19, no. 6, pp. 593-601.

Kottilil, S., Jackson, J.O. & Polis, M.A. (2005) 'Hepatitis B & hepatitis C in HIV-infection', *Indian Journal of Medical Research,* vol. 121, no. 4, pp. 424-450.

Kožíšek, M., Lepšík, M., Grantz Šašková, K., Brynda, J., Konvalinka, J. & Řezáčová, P. (2014) 'Thermodynamic and structural analysis of HIV protease resistance to darunavir–analysis of heavily mutated patient-derived HIV-1 proteases', *FEBS Journal*, vol. 281, no. 7, pp. 1834-1847.

Lall, M., Gupta, R., Sen, S., Kapila, K., Tripathy, S. & Paranjape, R. (2008) 'Profile of primary resistance in HIV-1-infected treatment-naïve individuals from Western India', *AIDS Research and Human Retroviruses*, vol. 24, no. 7, pp. 987-990.

Lambert, L. (2007) 'HIV and development challenges in Yemen: which grows fastest?', *Health Policy Plan*, vol. 22, no. 1, pp. 60-62.

Lengauer, T., Pfeifer, N. & Kaiser, R. (2014) 'Personalized HIV therapy to control drug resistance', *Drug Discovery Today*: Technologies, vol. 11, no. 0, pp. 57-64.

Lessells, R.J., Avalos, A. & de Oliveira, T. (2013) 'Implementing HIV-1 genotypic resistance testing in antiretroviral therapy programs in Africa: needs, opportunities, and challenges', *AIDS Reviews*, vol. 15, no. 4, pp. 221-229.

Liu, T.F. & Shafer, R.W. (2006) 'Web resources for HIV type 1 genotypic-resistance test interpretation', *Clinical Infectious Disease*s, vol. 42, no. 11, pp. 1608-1618.

Loi, P., Modlinski, J. & Ptak, G. (2011) 'Interspecies somatic cell nuclear transfer: a salvage tool seeking first aid', *Theriogenology*, vol. 76, no. 2, pp. 217-228.

Madani, T.A. (2009) 'Hepatitis C virus infections reported over 11 years of surveillance in Saudi Arabia', *Transactions of the Royal Society of Tropical Medicine and Hygiene*, vol. 103, no. 2, pp. 132-136.

Madani, T.A., Al-Mazrou, Y.Y., Al-Jeffri, M.H. & Al Huzaim, N.S. (2004) 'Epidemiology of the human immunodeficiency virus in Saudi Arabia; 18-year surveillance results and prevention from an Islamic perspective', *BMC Infectious Diseases*, vol. 4, p. 25.

Maek-a-nantawat, W. (2014) Factors associated with syphilis acquisition among HIV-infected MSM on antiretroviral therapy, *2014 National STD Prevention Conferenc*e, CDC.

Maguire, H., Dale, J.W., McHugh, T.D., Butcher, P.D., Gillespie, S.H., Costetsos, A., Al-Ghusein, H., Holland, R., Dickens, A., Marston, L., Wilson, P., Pitman, R., Strachan, D., Drobniewski, F.A. & Banerjee, D.K. (2002) 'Molecular epidemiology of tuberculosis in London 1995-7 showing low rate of active transmission', *Thorax*, vol. 57, no. 7, pp. 617-622.

Marks, M., Solomon, A.W. & Mabey, D.C. (2014) 'Endemic treponemal diseases', *Transactions of The Royal Society of Tropical Medicine and Hygiene*, vol. 108, no. 10, pp. 601-607.

Malekinejad, M., Mohraz, M., Razani, N., Akbari, G., McFarland, W., Khairandish, P., Malekafzali, H., Gouya, M., Zarghami, A. & Rutherford, G. (2015) 'High HIV Prevalence in a Respondent-Driven Sampling Survey of Injection Drug Users in Tehran, Iran', *AIDS and Behavior*, vol. 19, no. 3, pp. 440-449.

May, M.T., Gompels, M., Delpech, V., Porter, K., Orkin, C., Kegg, S., Hay, P., Johnson, M., Palfreeman, A. & Gilson, R. (2014) 'Impact on life expectancy of HIV-1 positive individuals of CD4+ cell count and viral load response to antiretroviral therapy', *AIDS* (London, England), vol. 28, no. 8, p. 1193.

Mayer, K.H., Hanna, G.J. & Richard, T. (2001) 'Clinical use of genotypic and phenotypic drug resistance testing to monitor antiretroviral chemotherapy', *Clinical Infectious Diseases*, vol. 32, no. 5, pp. 774-782.

Mayers, D. (1996) 'Rational approaches to resistance: nucleoside analogues', *AIDS*, vol. 10 Suppl 1, pp. S9-13.

Medrano, J., Resino, S., Vispo, E., Madejon, A., Labarga, P., Tuma, P., Martín-Carbonero, L., Barreiro, P., Rodriguez-Novoa, S. & Jiménez-Nacher, I. (2011) 'Hepatitis C virus (HCV) treatment uptake and changes in the prevalence of HCV genotypes in HIV/HCV-coinfected patients', *Journal of Viral Hepatitis*, vol. 18, no. 5, pp. 325-330.

Messina, J.P., Humphreys, I., Flaxman, A., Brown, A., Cooke, G.S., Pybus, O.G. & Barnes, E. (2015) 'Global distribution and prevalence of hepatitis C virus genotypes', *Hepatology*, vol. 61, no. 1, pp. 77-87.

Metzner, K.J., Giulieri, S.G., Knoepfel, S.A., Rauch, P., Burgisser, P., Yerly, S., Gunthard, H.F. & Cavassini, M. (2009) 'Minority quasispecies of drug-resistant HIV-1 that lead to early therapy failure in treatment-naïve and-adherent patients', *Clinical Infectious Diseases*, vol. 48, no. 2, pp. 239-247.

Mirzazadeh, A., Emmanuel, F., Gharamah, F., Al-Suhaibi, A.H., Setayesh, H., McFarland, W. & Haghdoost, A.A. (2014) 'HIV prevalence and related risk behaviors in men who have sex with men, Yemen 2011', *AIDS and Behavior*, vol. 18, no. 1, pp. 11-18.

Moatti, J.P., Spire, B. & Kazatchkine, M. (2004) 'Drug resistance and adherence to HIV/AIDS antiretroviral treatment: against a double standard between the north and the south', *AIDS*, vol. 18 Suppl 3, pp. S55-61.

Mor, Z., Lidji, M., Cedar, N., Grotto, I. & Chemtob, D. (2013) 'Tuberculosis incidence in HIV/AIDS patients in Israel, 1983–2010', *PloS One*, vol. 8, no. 11, p. e79691.

Mocroft, A., Ledergerber, B., Viard, J.P., Staszewski, S., Murphy, M., Chiesi, A., Horban, A., Hansen, A.B., Phillips, A.N. & Lundgren, J.D. (2004) 'Time to virological failure of 3 classes of antiretrovirals after initiation of highly active antiretroviral therapy: results from the EuroSIDA study group', *Journal of Infectious Diseases*, vol. 190, no. 11, pp. 1947-1956.

Moore, E., Beadsworth, M.B., Chaponda, M., Mhango, B., Faragher, B., Njala, J., Hofland, H.W., Davies, J., Hart, I.J., Beeching, N.J., Zijlstra, E.E. & van Oosterhout, J.J. (2010) 'Favourable one-year ART outcomes in adult Malawians with hepatitis B and C co-infection', *Journal of Infection*, vol. 61, no. 2, pp. 155-163.

MSAC application 1067 (2009) *Genotypic resistance testing of antiretrovirals in HIV* [Online], © Commonwealth of Australia 2005, Available from: http://www.msac.gov.au/internet/msac/publishing.nsf/Content/BCDC2A9D05A33761CA2575AD0082FD32/$File/1067%20-%20Genotypic%20resistance%20testing%20of%20antiretrovirals%20in%20HIV%20Report.pdf (Accessed: 2 May 2015 at 15:00).

Mumtaz, G., Hilmi, N., McFarland, W., Kaplan, R.L., Akala, F.A., Semini, I., Riedner, G., Tawil, O., Wilson, D. & Abu-Raddad, L.J. (2011) 'Are HIV epidemics among men who have sex with men emerging in the Middle East and North Africa?: a systematic review and data synthesis', *PLoS Medicine*, vol. 8, no. 8, p. e1000444.

Mutlu, E., (2014) *HIV epidemiology in Turkey* [Online], European Monitoring Centre for Drugs and Drug Addition, Available from: www.file://ufs01/user01/WAIL2010/Documents/05.%20E.%20Mutlu%20-%20HIV%20epidemilogy%20Turkey.pdf (Accessed: 11.5.2015 at 13:00).

Muula, A. S., Thomas, J. C., Pettifor, A. E., Strauss, R. P., Suchindran, C. M., & Meshnick, S. R. (2012). Religion is not associated with HIV infection among women in Malawi. *International Journal on Disability and Human Development*, vol. 11, no. 2, pp. 121-131.

Naba, M.R., Kanafani, Z.A., Awar, G.N. & Kanj, S.S. (2010) 'Profile of opportunistic infections in HIV-infected patients at a tertiary care center in Lebanon', *Journal of Infection and Public Health*, vol. 3, no. 3, pp. 130-133.

National AIDS Program. NAP, (2014) Saudi National AIDS programme policy and guidelines [Online] avsilable from: http://www.napksa.com/program.php#Policy (Accessed: 5 May 2015 at 15:00).

National AIDS Trust. NAT (2012).'Hepatitis C and HIV Coinfection' Report January 2012, available at: http://www.nat.org.uk/media/Files/Publications/Jan-2012-Hepatitis-C-and-HIV-co-infection.pdf [accessed 27 April 2015]

National Statistical Services. NSS, (2012) Sample size calculater [Online] Available from: http://www.nss.gov.au/nss/home.nsf/NSS/0A4A642C712719DCCA2571AB00243DC6?opendocument (Accessed: 14 May 2015 at 18:00).

Nicoll, A., Gill, O.N., Peckham, C.S., Ades, A., Parry, J., Mortimer, P., Goldberg, D., Noone, A., Bennett, D. & Catchpole, M. (2000) 'The public health applications of unlinked anonymous seroprevalence monitoring for HIV in the United Kingdom', *International Journal of Epidemiology*, vol. 29, no. 1, pp. 1-10.

Njoh, J., & Zimmo, S. (1997). The prevalence of human immunodeficiency virus among drug-dependent patients in Jeddah, Saudi Arabia. *Journal of substance abuse treatment*, vol.14, no. 5, pp.487-488.

Novak, R.M., Chen, L., MacArthur, R.D., Baxter, J.D., Hullsiek, K.H., Peng, G., Xiang, Y., Henely, C., Schmetter, B. & Uy, J. (2005) 'Prevalence of antiretroviral drug resistance mutations in chronically HIV–infected, treatment-naïve patients: implications for routine resistance screening before initiation of antiretroviral therapy', *Clinical Infectious Diseases*, vol. 40, no. 3, pp. 468-474.

Obermeyer, C.M. (2006) 'HIV in the Middle East', *BMJ*, vol. 333, no. 7573, pp. 851-854.

Omair, M.A., Al-Ghamdi, A.A. & Alrajhi, A.A. (2010) 'Incidence of tuberculosis in people living with the human immunodeficiency virus in Saudi Arabia', *Int Journal of Tuberculosis and Lung Disease*, vol. 14, no. 5, pp. 600-603.

Palella, F.J., Jr., Delaney, K.M., Moorman, A.C., Loveless, M.O., Fuhrer, J., Satten, G.A., Aschman, D.J. & Holmberg, S.D. (1998) 'Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators', *New England Journal of Medicine*, vol. 338, no. 13, pp. 853-860.

Paredes, R., Mocroft, A., Kirk, O., Lazzarin, A., Barton, S.E., van Lunzen, J., Katzenstein, T.L., Antunes, F., Lundgren, J.D. & Clotet, B. (2000) 'Predictors of virological success and ensuing failure in HIV-positive patients starting

highly active antiretroviral therapy in Europe: results from the EuroSIDA study', *Archives of Internal Medicine*, vol. 160, no. 8, pp. 1123-1132.

Perez-Elias, J., Garcia-Arota, I., Muñoz, V., Santos, I., Sanz, J., Abraira, V., Arribas, J.R., González, J., Moreno, A. & Dronda, F. (2003) 'Phenotype or virtual phenotype for choosing antiretroviral therapy after failure: a prospective, randomized study', *Antiviral Therapy*, vol. 8, no. 6, pp. 577-584.

Peters, P.J., Marston, B.J., De Cock, K.M. (2013) HIV epidemiology in the Tropics. Chapter 5.9. Eds: Peters, J., Hotez, P., Junghanss, T., Kang, G., Lalloo, D. & White, N.J. eds.*Manson's Tropical Diseases: Expert Consult-Online.* Elsevier Health Sciences, pp 68-78

Pieniazek, D., Baggs, J., Hu, D.J., Matar, G.M., Abdelnoor, A.M., Mokhbat, J.E., Uwaydah, M., Bizri, A.R., Ramos, A. & Janini, L.M. (1998) 'Introduction of HIV-2 and multiple HIV-1 subtypes to Lebanon', *Emerging Infectious Diseases*, vol. 4, no. 4, p. 649.

Piot, P., Bartos, M., Ghys, P.D., Walker, N. & Schwartlander, B. (2001) 'The global impact of HIV/AIDS', *Nature*, vol. 410, no. 6831, pp. 968-973.

Porco, T.C., Lewis, B., Marseille, E., Grinsdale, J., Flood, J.M. & Royce, S.E. (2006) 'Cost-effectiveness of tuberculosis evaluation and treatment of newly-arrived immigrants', *BMC Public Health*, vol. 6, p. 157.

Pozniak, A., Gazzard, B., Anderson, J., Babiker, A., Churchill, D., Collins, S., Fisher, M., Johnson, M., Khoo, S., Leen, C., Loveday, C., Moyle, G., Nelson, M., Peter, B., Phillips, A., Pillay, D., Wilkins, E., Williams, I. & Youle, M. (2003) 'British HIV Association (BHIVA) guidelines for the treatment of HIV-infected adults with antiretroviral therapy', *HIV Medicine*, vol. 4 Suppl 1, pp. 1-41.

Post, F.A., Grint, D., Werlinrud, A.M., Panteleev, A., Riekstina, V., Malashenkov, E.A., Skrahina, A., Duiculescu, D., Podlekareva, D., Karpov, I., Bondarenko, V., Chentsova, N., Lundgren, J., Mocroft, A., Kirk, O. & Miro, J.M. (2014) 'Multi-drug-resistant tuberculosis in HIV positive patients in Eastern Europe', *Journal of Infection*, vol. 68, no. 3, pp. 259-263.

Puls, R.L., Srasuebkul, P., Petoumenos, K., Boesecke, C., Duncombe, C., Belloso, W.H., Molina, J.-M., Li, L., Avihingsanon, A. & Gazzard, B. (2010) 'Efavirenz versus boosted atazanavir or zidovudine and abacavir in antiretroviral treatment-naïve, HIV-infected subjects: week 48 data from the Altair study', *Clinical Infectious Diseases*, vol. 51, no. 7, pp. 855-864.

Rahimi-Movaghar, A., Amin-Esmaeili, M., Haghdoost, A.-a., Sadeghirad, B. & Mohraz, M. (2012) 'HIV prevalence amongst injecting drug users in Iran: A systematic review of studies conducted during the decade 1998–2007', *International Journal of Drug Policy*, vol. 23, no. 4, pp. 271-278.

Rhee, S.-Y., Taylor, J., Fessel, W.J., Kaufman, D., Towner, W., Troia, P., Ruane, P., Hellinger, J., Shirvani, V. & Zolopa, A. (2010) 'HIV-1 protease mutations and protease inhibitor cross-resistance', *Antimicrobial Agents and Chemotherapy*, vol. 54, no. 10, pp. 4253-4261.

Riedner, G. & Dehne, K.L. (1999) 'HIV/AIDS surveillance in developing countries. Experiences and issues'.

Rodriguez-Rosado, R., Briones, C. & Soriano, V. (1999) 'Introduction of HIV drug-resistance testing in clinical practice', *AIDS*, vol. 13, no. 9, pp. 1007-1014.

Romanelli, F. & Pomeroy, C. (2000) 'Human immunodeficiency virus drug resistance testing: state of the art in genotypic and phenotypic testing of antiretrovirals', *Pharmacotherapy*, vol. 20, no. 2, pp. 151-157.

Ross, C.E., Tao, G., Patton, M. & Hoover, K.W. (2015) 'Screening for human immunodeficiency virus and other sexually transmitted diseases among US women with prenatal care', *Obstetrics & Gynecology*, vol. 125, no. 5, pp. 1211-1216.

Saad, M.D., Al-Jaufy, A., Grahan, R.R., Nadai, Y., Earhart, K.C., Sanchez, J.L. & Carr, J.K. (2005) 'HIV type 1 strains common in Europe, Africa, and Asia cocirculate in Yemen', *AIDS Research & Human Retroviruses*, vol. 21, no. 7, pp. 644-648.

Sabin, C.A., Hill, T., Lampe, F., Matthias, R., Bhagani, S., Gilson, R., Youle, M.S., Johnson, M.A., Fisher, M., Scullard, G., Easterbrook, P., Gazzard, B. & Phillips, A.N. (2005) 'Treatment exhaustion of highly active antiretroviral therapy (HAART) among individuals infected with HIV in the United Kingdom: multicentre cohort study', *BMJ*, vol. 330, no. 7493, p. 695.

Saeed, N., Farid, E. & Jamsheer, A.E. (2014) 'Opportunistic infections in HIV positive patients in Bahrain in 4 years study 2009-2013', *BMC Infectious Diseases*, vol. 14, no. 2, pp. 1-1.

Sayan, M., Willke, A., Ozgunes, N. & Sargın, F. (2013) 'HIV-1 subtypes and primary antiretroviral resistance mutations in antiretroviral therapy naïve HIV-1 infected individuals in Turkey', *Japanese Journal of Infectious Diseases*, vol. 66, no. 4, pp. 306-311.

Seal, D. (2013) HIV and STI service provision in Syria: A study of contradictions, 141st APHA Annual Meeting and Exposition (November 2-November 6, 2013), APHA.

Scrimgeour, E.M., Mehta, F.R. & Suleiman, A.J. (1999) 'Infectious and tropical diseases in Oman: a review', *American Journal of Tropical Medicine and Hygiene*, vol. 61, no. 6, pp. 920-925.

SeyedAlinaghi, S., Jam, S., Mehrkhani, F., Fattahi, F., Sabzvari, D., Kourorian, Z., Jabbari, H. & Mohraz, M. (2011) 'Hepatitis-C and hepatitis-B co-infections in patients with human immunodeficiency virus in Tehran, Iran', *Acta Medica Iranica*, vol. 49, no. 4, pp. 252-257.

Shawky, S., Soliman, C. & Sawires, S. (2009) 'Gender and HIV in the Middle East and North Africa: lessons for low prevalence scenarios', *Journal of Acquired Immune Deficiency Syndrome*, vol. 51, Suppl. 3, pp. S73-74.

Shekelle, P., Maglione, M., Geotz, M.B., Wagner, G., Wang, Z., Hilton, L., Carter, J., Chen, S., Tringle, C., Mojica, W. & Newberry, S. (2007) 'Antiretroviral (ARV) drug resistance in the developing world', *Evidence Report Technology Assess essment*, no. 156, pp. 1-74.

Sirivichayakul, S., Kantor, R., DeLong, A.K., Wongkunya, R., Mekprasan, S., Ruxrungtham, K., Sohn, A.H. & Phanuphak, P. (2014) 'Transmitted HIV drug resistance at the Thai Red Cross Anonymous Clinic in Bangkok: results from three consecutive years of annual surveillance', *Journal of Antimicrobial Chemotherapy*, vol. 10 no. 4 p. 499.

Sterling, R.K., Wegelin, J.A., Smith, P.G., Stravitz, R.T., Luketic, V.A., Fuchs, M., Puri, P., Shiffman, M.L., Contos, M.A., Mills, A.S. & Sanyal, A.J. (2010) 'Similar progression of fibrosis between HIV/HCV–infected and HCV–infected patients: analysis of paired liver biopsy samples', *Clinical Gastroenterology and Hepatology*, vol. 8, no. 12, pp. 1070-1076.

Stone, V., Ojikutu, B., Rawlings, M.K. & Smith, K. (2009) *HIV/AIDS in US Communities of Color,* Springer.

Sufian, S. (2004) 'HIV/AIDS in the Middle East and North Africa: a primer', *Middle East Report,* pp. 6-9.

Tanimura, T., Jaramillo, E., Weil, D., Raviglione, M. & Lönnroth, K. (2014) 'Financial burden for tuberculosis patients in low-and middle-income countries: a systematic review', European Respiratory Journal, vol. 43, no. 6, pp. 1763-1775.

Temereanca, A., Ene, L., Mehta, S., Manolescu, L., Duiculescu, D. & Ruta, S. (2013) 'Transmitted HIV drug resistance in treatment-naïve Romanian patients', *Journal of Medical Virology*, vol. 85, no. 7, pp. 1139-1147.

Tisdale, M., Kemp, S.D., Parry, N.R. & Larder, B.A. (1993) 'Rapid in vitro selection of human immunodeficiency virus type 1 resistant to 3'-thiacytidine

inhibitors due to a mutation in the YMDD region of reverse transcriptase', *Proceedings of the National Academy of Sciences USA*, vol. 90, no. 12, pp. 5653-5656.

Traboulsi, R., Kanafani, Z.A., Nakib, M. & Kanj, S.S. (2006) 'Epidemiology of HIV infection in Lebanon. Data from 1985-2005', *Le Journal Médical Libanais*, vol. 54, no. 2, pp. 61-64.

University of California San Francisco. UCSF (2010).'*Hepatitis B and HIV Coinfection*' October 2010, available at: http://hivinsite.ucsf.edu/InSite?page=kb-05-03-04#S2X [accessed 26 April 2015]

Vallecillo, G., Mojal, S., Torrens, M. & Muga, R. (2014) Antiretroviral therapy (ART) Use, Human Immunodeficiency Virus (HIV)-1 RNA suppression, and medical causes of hospitalization among HIV-infected intravenous drug users in the late ART era, *Open Forum Infectious Diseases*, vol. 1, Oxford University Press, p. ofu010.

Walmsley, S. (2007) 'Protease inhibitor-based regimens for HIV therapy: safety and efficacy', *Journal of Acquired Immune Deficiency Syndromes*, vol. 45, pp. S5-S13.

Wensing, A.M., Calvez, V., Günthard, H.F., Johnson, V.A., Paredes, R., Pillay, D., Shafer, R.W. & Richman, D.D. (2014) '2014 update of the drug resistance mutations in HIV-1', *Topics in Antiviral Medicine*, vol. 22, no. 3, pp. 642-650.

Wiebe, E.R., Comay, S.E., McGregor, M. & Ducceschi, S. (2000) 'Offering HIV prophylaxis to people who have been sexually assaulted: 16 months' experience in a sexual assault service', *Canadian Medical Association Journal,* vol. 162, no. 5, pp. 641-645.

Williams, I., Churchill, D., Anderson, J., Boffito, M., Bower, M., Cairns, G., Cwynarski, K., Edwards, S., Fidler, S. & Fisher, M. (2014) 'British HIV Association guidelines for the treatment of HIV-1-positive adults with antiretroviral therapy 2012 (Updated November 2013) ', *HIV Medicine*, vol. 15, no. S1, pp. 1-6.

World Health Organization. WHO (2007) *Case Definitions of HIV for Surveillance and Revised Clinical Staging and Immunological Classification of HIV-Related Disease in Adults and Children* 2007.

World Health Organization. (2007) *Global tuberculosis control: epidemiology, strategy, financing: WHO report 2007*, World Health Organization.

World Health Organization. (2009). *UN Joint Programme on HIV/AIDS, Global Report: UNAIDS Report on the Global AIDS Epidemic:*

2012, ISBN 978-92-9173-996-7, available at: http://www.unaids.org/en/media/unaids/contentassets/documents/epidemiology/2012/gr2012/20121120_UNAIDS_Global_Report_2012_en.pdf [accessed 26 February 2013]

World Health Organization. (2009) *Global tuberculosis control: epidemiology, strategy, financing: WHO report 2009*, World Health Organization.

World Health Organization. (2010) '*WHO guidelines on drawing blood: best practices in phlebotomy*'.

World Health Organization. (2012). *Global Alert and Response: HBV report 2012,* available at: http://www.who.int/csr/disease/hepatitis/whocdscsrlyo20022/en/index1.html#where [accessed 26 May 2015]

World Health Organization. (2013). *HIV surveillance in the WHO Eastern Mediterranean Region: regional update 2012.*

World Health Organization. (2013). *UN Joint Programme on HIV/AIDS, Global Report: UNAIDS Report on the Global AIDS Epidemic*: 2012, ISBN 978-92-9173-996-7, available at: http://www.unaids.org/en/media/unaids/contentassets/documents/epidemiology/2012/gr2012/20121120_UNAIDS_Global_Report_2012_en.pdf [accessed 26 February 2013]

Vella, S. & Palmisano, L. (2005) 'The global status of resistance to antiretroviral drugs', *Clinical Infectious Diseases*, vol. 41 Suppl 4, pp. S239-246.

Yerly, S., Rakik, A., Kinloch-de-Loes, S., Erb, P., Vernazza, P., Hirschel, B. & Perrin, L. (1996) '[Prevalence of transmission of zidovudine-resistant viruses in Switzerland. l'Etude suisse de cohorte VIH]', *Schweizerische Medizinische Wochenschrift,* vol. 126, no. 43, pp. 1845-1848.

Young, B., Carmichael, J.K., Johnson, D. & Mills, T. (2006) 'PA update: DHHS guidelines for the treatment of HIV infection', *Journal of the American Academy of Physician Assistants*, vol. Suppl, pp. 3-13.

Zarocostas, J. (2009) 'WHO revises upwards estimated number of TB deaths associated with HIV', *BMJ*, vol. 338, p. b1253.

Zohar, M., Moshe, L., Daniel, C., Noa, C. & Itamar, G. (2014) 'HIV prevalence in the Israeli tuberculosis cohort, 1999-2011', *BMC Public Health*, vol. 14, no. 1, p. 1090.

Appendix A

Patients log. During data collection (Clinical features and epidemiology of HIV/AIDS in KSA)

#	Code				Name				File #	DOB	Sex	Age	Date
	Let.	Numbers			1st	2nd	family	Mother's name					
1													
2													
3													
4													
5													
6													
7													
8													
9													
10													
11													
12													
13													
14													
15													
16													
17													
...													

Dr.Wail bajhmom

Dr.Nick Beeching 16.07.2010

Appendix B

Data collection sheet(Clinical Features and Epidemiology HIV KSA)

Section	Patient's				Remarks
A	Data		Code: Letter	Numbers	
	Age	A.( )18-25 B. ( )26-35	C.( )36-45	D.( )46-60	E.( )60+
	Sex	1. ( )female	2. ( )Male		
	Education	A.( )Illiterate B( ).R&W	C.( )High School	D.( ) University	E.( )PG
	Marital Status	1.( )Single	2.( )Married	3.( )Divorced	
		4.( )Widow			
section					
B	History				
	HIV Dx date	Duration A.( ) 1y or less	B.( )2-5y C.( )5-10	D.( )>10y	
		Age at Dx ( )years			
	Presenting history				
	HIV Dx	Clinical suspicion 1.( )yes. 2.( ) no	Pre-marital test 1.( )yes 2.( ) no	Routine serology 1.( )yes 2.( )no	
		pre-occupation 1.( )yes 2.( ) no			
	History of	1.( )Hetero sexuality	2.( )Homosexuality	3.( )IDU	
		4.( )Blood Transfusion	5.( ) Unprotected sex	6.( )Contact w/ HIV	
		7.( ) History of prison	8.( )STI	9.( )Psychotic history.	
		10.( ) travel history			
	Co infections	HBV A.( )yes B.( )no C.( )not tested	HCV A.( ) yes B.( )no C. ( )ND	TB A.( )yes B ( )no C. ( )treated D. ( )not treated	
section	Lab.				
C		WBC			
		Hb			

		CD4+ %	Absolute	CD8 + count	
		Lymphocyte %	Absolute		
		Esinophils %	Absolute		
		HBsAg 1. ( )yes 2.( )no 3.( )ND	HCV Ag (PCR) 1.( )yes .( ) no 3.( )ND	Toxo 1.( )yes 2.( ) no 3.( ) ND	
		CMV 1.( ) yes 2.( ) no 3.( ) ND	Cryptococcal 1.( )yes 2.( )no 3.( )ND	EBV 1.( )yes 2.( )no 3.( )ND	
		VZV 1.( )yes 2.( )no 3.( )ND	VDRL 1.( )yes 2.( )no 3.( )ND	Other STI	
		Viral Load:			
		Log	Copies /ml		

	CDC/WHO Staging at presentation	A.( ) stage 1	B.( ) stage 2		
		C.( ) stage 3	D. ( ) stage 4		
section	Medication				
D	ART Used in the Past	1. ( )Yes	2. ( )No		
	Regimen				
	ART resistance	A. ( )Done	B.( )ND		
	Resistance detected	A. ( )Yes	B.( )No		
	Currently on ART	1. ( )Yes	2. ( )No		
	Regimen				
	ART resistance	A. ( )Done	B.( )ND		
	Resistance detected	A. ( )Yes	B.( )No		

	Latter use of ART	**1. ( )Yes**	**2. ( )No**		
	Regimen				
	ART resistance	**A. ( )Done**	**B.( )ND**		
	Resistance detected	**A. ( )Yes**	**B.( )No**		

Dr.Wail Bajhmom
Dr. Nick Beeching 16.07.2010

Appendix C

Dr.Bajhmom
Consent (ART resistance Jeddah) 2012

CONSENT	**اقرار بالموافقة**
I_____	**أنا_____**
I understand and consent to take extra samples of my blood. I acknowledge that these samples will be stored for research purposes (ART resistance).	**أتفهم واعطي موافقتي لاخذ عينة اضافية من دمي. كما انني على علم تام بأن هذه العينة سوف تخزن وتستخم لغرض البحث العلمي(مقاومة المضادات الفيروسية)**
I consent to this sample to be used to provide extra information to my doctor at the clinic to guide the best treatment to myself.	**وستعطي معلومات للطبيبي المعالج وتساعد لحصولي لافضل علاج ممكن وعليه اوافق على استخدام وتحليل هذه العينة لكل ماله علاقة بالدراسة.**
I understand that the samples will be sent to Liverpool UK for analysis related to the research at the University labs.	**وانا على علم بأن هذه العينة سترسل الى ليفربول بالمملكة المتحدة لعمل الاختبارات اللازمة في مختبرات الجامعة.**
I understand that confidentiality will be assured and I will not be identified if the results are published.	**وقد ا وقد تم التاكيد على سرية الدراسة وأنه لن يتم التعرف على شخصيتي في حال نشر النتائج.**
Signature of participant:_____	**توقيع المشارك_____**
Signature of interviewer (witness):_____	**توقيع المقابل(الشاهد)_____**
Date _____.	**التاريخ_____**

Appendix D

Questionnaire (data collection ART resistance)

#	Pt code								
Age									
Sex	1.( ) male			2.( ) female					
HIV duration:									
On ART	1.( ) yes			2.( ) no					
Adherence	1.poor ( )			2.reasonable ( )			**3.Excellent ( )**		
Duration of ART	1.( )< 6 months			2. ( )>6 months					
Total years									
ART regimen									
How many different regimen	0	1		2	more		**Unknown**		
	A. (NRTI)	now	previous	**B**. (NNRTI)	now	previous	**C. (PI)**	now	**Previous**
	1. Zidovudine ZDV			1. efavirenz EFV			1.Atazanavir, ATV		
	2. Lamivudine 3TC			2.nevirapine, NVP			2. Lopinavir LPV		
	3. Combivir			3.etravirine			3.Ritonavir RTV		
	4.Tenofovir			4.			4.Kalerta		
	5.Emtricitabine FTC								
	6.Truvada								
CD4+				**Viral load**					
	Absolute	%					Absolute	**Log**	
Nadir					Nadir				
Peak					**Peak**				

Dr.Bajhmom (ART resistance Jeddah) 2012

Appendix E (Ethical approvals)

Dr Wail Bajhmoum
Liverpool School of Tropical Medicine
Pembroke Place
Liverpool
L3 5QA

LSTM
LIVERPOOL SCHOOL OF TROPICAL MEDICINE

Pembroke Place,
Liverpool, L3 5QA, UK
Tel: +44 (0)151 705 3100
Fax: +44 (0)151 705 3370

www.liv.ac.uk/lstm

Thursday, 04 November 2010

Dear Dr Wail Bajhmoum

Re: Research Protocol (10.66) Clinical and laboratory features of HIV/AIDS in the Kingdom of Saudi Arabia

Thank you for your letter dated 29 October 2010 responding to the points raised by the Research Ethics Committee. The protocol now has formal ethical approval from the Chair of LSTM Research Ethics Committee.

The approval is for a fixed period of three years, renewable annually thereafter. The committee may suspend or withdraw ethical approval at any time if appropriate.

Approval is conditional upon:

- Submission of ethical approval from other ethics committees.
- Notification of all amendments to the protocol for approval before implementation.
- Notification of when the project actually starts.
- Provision of an annual update to the Committee. Failure to do so could result in suspension of the study without further notice.
- Reporting of all severe unexpected Adverse Events to the Committee
- Reporting of new information relevant to patient safety to the Committee
- Provision of Data Monitoring Committee reports (if applicable) to the Committee

Failure to comply with these requirements will result in withdrawal of approval. The Committee would also like to receive copies of the final report once the study is completed.

Yours sincerely

Prof David Lalloo
Deputy Chair, Research Ethics Committee

Wail Bajhmom
Liverpool School of Tropical Medicine
Pembroke Place
Liverpool
L3 5QA

LSTM
LIVERPOOL SCHOOL
OF TROPICAL MEDICINE

Pembroke Place,
Liverpool, L3 5QA, UK
Tel: +44 (0)151 705 3100
Fax: +44 (0)151 705 3370

www.liv.ac.uk/lstm

Thursday, 09 August 2012

Dear Wail Bajhmom

Re: Research Protocol (12.15) Clinical and laboratory features of HIV/AIDS in the Kingdom of Saudi Arabia (Phase 2) – ART Resistance in Saudi HIV patients in Jeddah

Thank you for your letter dated 6 August 2012 responding to the points raised by the Research Ethics Committee. The protocol now has formal ethical approval from the Co-Chair of LSTM Research Ethics Committee.

The approval is for a fixed period of three years, or for the duration of the grant, renewable annually thereafter. The committee may suspend or withdraw ethical approval at any time if appropriate.

Approval is conditional upon:

- Submission of ethical approval from other ethics committees.
- Notification of all amendments to the protocol for approval before implementation.
- Notification of when the project actually starts.
- Provision of an annual update to the Committee. Failure to do so could result in suspension of the study without further notice.
- Reporting of all severe unexpected Adverse Events to the Committee
- Reporting of new information relevant to patient safety to the Committee
- Provision of Data Monitoring Committee reports (if applicable) to the Committee

Failure to comply with these requirements will result in withdrawal of approval. The Committee would also like to receive copies of the final report once the study is completed.

Yours sincerely

Dr Brian Faragher
Co-Chair, Research Ethics Committee

cc. Nick Beeching

Copy of e-mail Ethical Approval from Saudi MOH

RE: 12-159 Clinical and laboratory features of HIV/AIDS in KSA

From: Omar H. Kasule <okasule@kfmc.med.sa> > > To: "zmemish@yahoo.com" <zmemish@yahoo.com> > > Cc: malakita al masri malouk22@hotmail.com
>> Cc: bajhmoum@hotmail.com
>> Cc: nbeeching@blueyonder.co.uk > > Sent: Tuesday, August 14, 2012 1:05 PM
> > Subject: **12-159 Clinical and laboratory features of HIV/AIDS in the Kingdom > > of Saudi Arabia (Antiretroviral Resistance in Jeddah)**
IRB Registration Number with KACST, KSA: H-01-R-012 > > IRB Registration Number with OHRP/NIH, USA : IRB00008644 > > Approval Number Federal Wide Assurance NIH , USA : FWA00018774 > > August 14, 2012 > > IRB > > Log Number: 12-159

I am pleased to inform you that your study titled: 'Clinical and > > laboratory features of HIV/AIDS in the Kingdom of Saudi Arabia (Antiretroviral Resistance in Jeddah)' > > was reviewed and was approved.

> > > > Please > > be informed that in conducting this study, you as the Principal Investigator > > are required to abide by the rules and regulations of the Government of > > Saudi Arabia > > and KFMC/IRB. Further, you are required to submit a Progress Report before > > July > > 14, 2013; it can be reviewed by the IRB without lapse of approval. The > > approval > > of this proposal will automatically be suspended on August 14, 2013 pending > > the > > acceptance of the Progress Report. You also need to notify the IRB as soon as possible in the case of:

1. Any amendments to the project; > > 2. Termination of the study; > > 3. Any serious unexpected adverse events (within two working days). > > 4. Any event or new information that may affect the benefit/risk ratio of > > the proposal. > > > > Please > > observe the following: > > > > 1. Personal identifying data should only be collected when necessary for > > research; > > 2. The data collected should only be used for this proposal; > > 3. Data should be stored securely so that a few authorized users are > > permitted access to the database; > > 4. Secondary disclosure of personal identifiable data is not allowed. > > 5. Copy of the Consent Form should be kept in the Research Subject's > > Medical Record and the consent process should be documented in the medical > > record. > > 6. Blood samples send overseas will not be used for any human genetic > > studies > > > > We > > wish you every success in your research endeavor. > > > > If > > you have any further questions feel free to contact me.

Sincerely

yours,
Prof. Omar H. Kasule
Chairman Institutional Review Board--IRB.
KingFahd Medical City,
Riyadh , KSA.
Tel: + 966 1 288 9999 Ext. 7540

Appendix F

List of presentations and publication

- LSTM presentation, Marriott Hotel, Liverpool Friday 14th May 2010 (poster)
- LSTM PG Presentation Day May 2011 (poster)
- University of Liverpool Presentation Day March 2011 (poster)
- LSTM PG presentation 21 May 2012 Liverpool (talk)
- British Infection Association 15th Annual Meeting Friday 25th May 2012 London (poster)
- Royal Society of Tropical Medicine and Hygiene Research in Progress 2012 poster presentation, at School of Oriental and African Studies (SOAS) London Dec 2012 (poster).
- LSTM postgraduate presentation day May 2013 (talk)
- LSTM postgraduates presentation (May 2013 and 2014))
- Both project phases were published in HIV Medicine volume 15 April 2014 and presented in third BHIVA and BASHH joint conference April 2014.

Clinical and laboratory features of HIV/AIDS in the Kingdom of Saudi Arabia

Dr Wail Bajhmoum PhD student
King Fahd General Hospital, Jeddah & Liverpool School of Tropical Medicine

Introduction:

•Globally, estimated 33 million people were living with HIV in 2007

• Overall, 2.0 million people died with AIDS in 2007

•There is little information about HIV in the Middle East (1,2)

HIV in Saudi Arabia:

• Saudi Arabia occupies most of the Arabian Peninsula.

•The population is 28,686,633 including 5,576,076 non-KSA (July 2009)

•Approximately 380 000 people were living with HIV in 2007

•Total new HIV cases in 2008 were 505 (34.6% annual increase from previous year 2007) (4).

•Most of the early cases acquired HIV 1 from blood product s and transfusion (Figure 1)

• Subsequently, the most prevalent mode of transmission became heterosexual (3)

• Male to female ratio is 5:1

•Jeddah region has the highest proportion of HIV cases in KSA (37%)

•Antiretroviral therapy (ART) is provided free of charge to all Saudi patients in new medical centers with up-to-date facilities

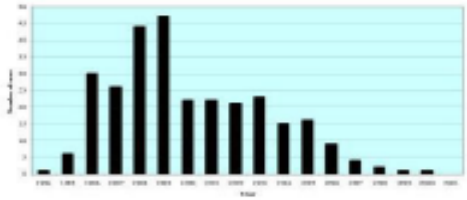


Figure 1. Number of HIV infections transmitted through blood transfusion from 1984 to 2001 (2)

Hypotheses:

•Common presenting syndromes of HIV patients in Jeddah are different from HIV patients in Europe and Sub-Sahara Africa

•TB and/or hepatitis co infection with HIV in Saudi patients is more common in patients who have been in prison.

•Patients who had previously experienced ART are more likely to develop resistance.

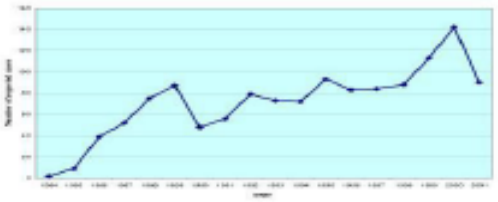


Figure 2. Annually reported HIV infections among Saudi citizens from 1984 through 2001 (2)

Methods:

* Retrospective case study of out patient clinics and inpatients in Jeddah

*HIV clinics for around 1200 HIV Saudi adults (18 years or older)

* Univariate and multivariate comparison of demographic and other features

* A cross-sectional study will be performed to evaluate the presence of ART resistance

* HIV resistance assay using genotypic test to detect antiretroviral resistance mutations in the HIV genome

* PCR products are sequenced and interpreted using Stanford HIV Drug Resistance Database(5)

Study aims:

* Review the literature on HIV/AIDS in the Middle East

*To describe the clinical features of HIV infection in adults in KSA in OPD and hospital admissions

* To describe prevalence and clinical features of co infection with hepatitis viruses and/or TB infection in HIV patients in Jeddah

* To document primary and secondary ART resistance rates and treatment outcomes for patients in Jeddah region

References

(1)-Obermeyer, C. M. (2008). HIV in the Middle East. *BMJ* 339(7678): 861-4.

(2)-Madani, T. A., Y. Y. Al-Mazrou, et al. (2004). Epidemiology of the human immunodefisienoy virus in Saudi Arabia; 18-year surveillanoe results and prevention from an Islamio perspeotive. *BMC Infect Dis* 4: 25.

(3)-Alrajhi, A. A. (2004). Human immunodefisienoy virus in Saudi Arabia. *Saudi Med J* 25(11): 1658-63.

(4)-*Saudi annual report on AIDS*, Ministry of Health,2008.

(5)-HIV drug resistanoe Stanford Database, *http://hivdb.stanford.edu*

Clinical and laboratory features of HIV/AIDS in the Kingdom of Saudi Arabia

W Bajhmoum[1,2] NJ Beeching[1]
1 Clinical Group, Liverpool School of Tropical Medicine, UK. 2 Ministry of Health, Kingdom of Saudi Arabia

Introduction

* There is little information about HIV in the Middle East [1,2]

* The Kingdom of Saudi Arabia (KSA) occupies most of the Arabian Peninsula and remains a low HIV prevalence country, with early cases acquired from blood product transfusion. Sexual transmission is now more important [3]

* The most recent available data show an increase of 505 cases nationally between 2007 & 2008 (increase of 34.6%). [4] Jeddah region has the highest proportion of cases (37%)

* Antiretroviral therapy (ART) is provided free of charge to all Saudi patients in medical centres with up-to-date facilities

Study aims

* To review the literature on HIV/AIDS in the Middle East

* To describe the clinical and demographic features of HIV infection in adults in Jeddah, KSA

* To describe prevalence and clinical features of co infection with hepatitis viruses and/or TB infection in HIV patients in Jeddah

Methods

* Retrospective case note review of all patients attending or admitted to King Saud Hospital (main referral centre) in Jeddah in 2010

* Extraction of clinical and demographic data on standardized, anonymised proformas

* Comparison of demographic and other features

* Subsequently, to determine the prevalence and patterns of ART resistance and treatment outcomes

Results

Results

* 1383 patients identified: median (range) age at HIV diagnosis was 35 (18-86) years

*1026 (75%) were male, 727 (52.6%) were married and 300 (27.4%) were illiterate

* 97 (7%) were IVDU and 98 (7%) had been in prison

* Only 12 (12%) of IVDU patients had been in prison (RR 1.97; 95% CI 1.04-3.75; p=0.058)

HIV testing and clinical features

* 795 (57%) were tested on clinical suspicion and 246 (17.8%) were diagnosed on routine premarital testing

* 611 (44%) had respiratory symptoms, 312 (22%) had GI symptoms and 408 (29%) no symptoms at diagnosis

* Only 122 (8.9%) were in WHO stages C or D at presentation

* 1026 (74%) are currently taking ART

Co-infection rates and key risks

	Prevalence	IVDU	Prison
Co-infection		RR (95% CI) P value	RR (95% CI) P value
TB	208/1383 (15%)	1.67 (1.13-2.41) 0.01	1.83 (1.18-2.85) 0.01
HCV	82/1101 (7.4%)	4.22 (2.71-6.57) <0.05	1.94 (1.04-3.63) 0.07
HBV (HBsAg pos)	59/1121 (5.3%)	1.89 (0.93-3.85) 0.08	2.38 (1.25-4.54) 0.01

Conclusions

* HIV demography and co-infection rates resemble many western cohorts

* Co-infections with TB and hepatitis C are correlated with difficult to reach groups

* The data have important and difficult implications for the HIV control and treatment programmes in KSA

* The next phase of the study will examine prevalence and molecular basis for ART resistance, about which there is very little information in the Region

References

1. Obermeyer CM. HIV in the Middle East. BMJ 2006; 333(7573): 851-4.
2. Madani TA, Al-Mazrou YY, et al. Epidemiology of the human immunodeficiency virus in Saudi Arabia; 15-year surveillance results and prevention from an Islamic perspective. BMC Infect Dis 2004; 4: 25.
3. Alraji, AA. Human immunodeficiency virus in Saudi Arabia. Saudi Med J 2004; 25(11): 1589-93.
4. Saudi Annual Report on AIDS 2008, Ministry of Health, 2008.

HIV resistance in ART naïve patients in a large treatment centre in Jeddah Kingdom of Saudi Arabia

W Bajhmoum[1,2] NJ Beeching[1] A Chawla[3] A Geretti[4] Z Memesh[2] M alsyed[2] M Hopkins[3]
1 Clinical Group, Liverpool School of Tropical Medicine, UK. 2 Ministry of Health, Kingdom of Saudi Arabia, 3 Liverpool Specialist Virology Centre, 4 Institute of Infection and Global Health, Liverpool

Introduction

* Only 2 publications about resistance to antiretroviral therapy (ART) in the Arabian Peninsula, including the Kingdom of Saudi Arabia (KSA) and they focus on high rate patterns with multiple failing regimens (1,2)
* The aim of this study was to determine patterns of ART resistance in a systematic fashion in treatment-naïve HIV positive Saudi patients and to document the presence and frequency of novel resistance HIV markers in Jeddah the largest treatment centre in KSA

Methods

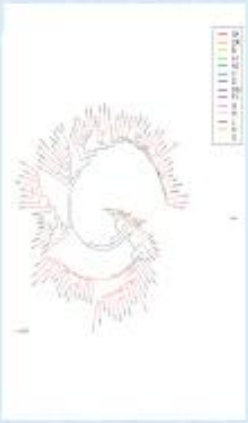
* Plasma samples were collected from all ART-naïve patients sequentially attending the HIV clinic at King Saud Hospital between November 2012 and February 2013
* HIV protease (Prot) and reverse transcriptase (RT) regions were sequenced according to routine in-house diagnostic protocols at Liverpool Specialist Virology Centre
* The Stanford database was used for interpretation of resistance profiles

NNRTI	Mutation	N (%)	Resistance to (ARV)
Total (10.3%)	E138A	3 (3.4)	RPV
	E138EG	2 (2.3)	ETR and RPV
	V179D	2 (2.3)	EVF and NVP
	V106M	1 (1.2)	EVF and NVP
	K103M	1 (1.2)	EVF and NVP
	K179E	1 (1.2)	EVF and NVP
	V106I	1 (1.2)	EVF and NVP
NRTI	Resistance	N (%)	Resistance to (ARV)
Total (1.1)	M184MV	1 (1.1)	3TC, ABC, DDI and FTC
PI	Resistance	N (%)	Resistance to (ARV)
Total (6.5%)	L33F	1 (1.1)	FPV/r and NFV
	K43T	1 (1.1)	NFV and TPV
	L10IL	2 (2.2)	NFV
	L10I	2 (2.2)	NFV

Results

* Blood samples were collected from 109/116 (94%) patients approached to join the study
* 71 (65%) were male and 52 (48%) had been diagnosed with HIV within the last 6 months
* Mutations at putative resistance sites were detected in 25/96 (26%)
* Potential to confer significant resistance to NNRTI was found in 9/87 (10.3%)
* Individual class drug resistance was found for NNRTI, (10.3%), NRTI (1.1%) and PI (6.5%)
* The main mutations are summarised in table 1
* 2 (2.3%) patients had mutations to Etravirine was found, although the drug is not in common use in KSA (table 1)
* The most common HIV-1 subtype was C (38%); although a cluster of CRF43_02G (21%) was also prominent

Phylogenetic tree



Conclusions

* Clinically significant primary resistance is already present in this population
* A variety of markers included some clustering suggesting local transmission of primary resistance
* The results are probably generalizable in KSA and we recommend the introduction of routine resistance testing for all HIV positive patients in the region before starting ART.

References
(1) Jamjoom, G. A., E. I. Azhar, et al. (2010). "Genotype and antiretroviral drug resistance of human immunodeficiency virus-1 in Saudi Arabia." Saudi Med J 31(9): 987-992.
(2) Al Dhahry, S. H., E. M. Scrimgeour, et al. (2004). "Human immunodeficiency virus type 1 infection in Oman: antiretroviral therapy and frequencies of drug resistance mutations." AIDS Res Hum