Analysis of HIV subtypes and the phylogenetic tree in HIV-positive samples from Saudi Arabia

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ABSTRACT

الأهداف: تحديد الأنواع الجينية المنتشرة لنقص المناعة المكتسب (HIV) في السعودية، وتحديد العلاقة الوراثية بينها في عينات موجبة مصليا للفيروس تم جمعها من المملكة العربية السعودية, ومقارنة هذه الأنواع بالأنواع المنتشرة في الدول الأخرى.

الطريقة: تم دراسة 39 عينة موجبة مصليا لفيروس نقص المناعة المكتسبة النوع الأول (HIV-1) وراثياً، بمدينة الدمام – المملكة العربية السعودية، باستخدام تقنية الجزيئات الحيوية وذلك بالتعاون مع مختبرات شركة ابوت الأمريكية خلال الفترة مابين 2004م وحتى 2007م.

النتائج: جميع العينات كانت مصابة بفيروس نقص المناعة المكتسب النوع الأول (HIV-1) من المجموعة M. من 39 عينة موجبة مصليا، 12 عينة كانت موجبة باستخدام تقنية البلمرة الجزيئية (PCR)، وكان النوع الجيني C الأكثر تواجداً، حيث وجد في 58% من العينات الموجبة، تلا ذلك النوع الجيني B في 17% من العينات، ثم الأنواع الجينية A وD وG حيث كانت نسبة التواجد 8% لكل منها. كما تم تحديد العلاقة الوراثية لكل الأنواع الموجودة.

خاتمة: تحديد الأنواع الجينية لفيروس نقص المناعة المكتسبة (HIV)، وعلاقتها الوراثية مهمة للدراسات الوبائية، كما يمكن أن تساعد في تحديد مصدر العدوي لفيروس نقص المناعة المكتسب (HIV).

Objective: To assess the prevalence of HIV-1 genetic subtypes in Saudi Arabia in samples that are serologically positive for HIV-1, and compare the HIV-1 genetic subtypes prevalent in Saudi Arabia with the subtypes prevalent in other countries.

Method: Thirty-nine HIV-1 positive samples were analyzed for HIV-1 subtypes using molecular techniques. The study is a retrospective study that was conducted in Dammam, Kingdom of Saudi Arabia, and in Abbott laboratories (United States of America) from 2004 to 2007.

Results: All samples were seropositive for HIV-1 group M. Of the 39 seropositive samples, only 12 were polymerase chain reaction positive. Subtype C is the most common virus strain as it occurred in 58% of these samples; subtype B occurred in 17%; and subtypes A, D and G were found in 8% each. The phylogenetic tree was also identified for the isolates.

Conclusion: Detection of HIV subtypes is important for epidemiological purposes and may help in tracing the source of HIV infections in the Kingdom of Saudi Arabia.

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There are currently 40 million individuals in the world infected with HIV,[1,2] and nearly 16,000 new infections occur worldwide each day based on the World Health Organization estimates. The search for an effective therapy and vaccine, to control the AIDS pandemic is still continuing long after the discovery and isolation of HIV, some 20 years ago.[3] This has been due to several unique challenges that HIV-1 has presented, which have prevented effective control of the virus. As HIV-1 continues to spread worldwide, its genetic diversification increases, fuelled by population movements and interactions.[4-7] Emergence of point mutations and recombination events are the mechanisms underlying HIV-1 genetic variability. Human immunodeficiency virus-1 strains are grouped into M, O, and N. Group M viruses radiated from Central Africa, and began their expansion in humans

approximately 70 years ago, and are responsible for the pandemic. Phylogenetic analyses identified 9 subtypes within group M, including A-D, F-H, J, and K, which are genetically equidistant, diverging by 20-30% in the *env* gene, and 15-22% in the *gag* gene.[8-10] Subtypes A and F include the sub-subtypes A1, A2, A3, A4, F1, and F2. Subtype D may be more appropriately classified as sub-subtype B2. At least 43 inter-subtype circulating recombinant forms (CRFs) have been identified. Additional unique recombinant forms have been recognized, and several have been characterized by full genome sequencing, the gold standard for subtype assignment. The diversity of HIV subtypes present a greater challenge for development of a universal AIDS vaccine, and may eventually affect the accuracy of the serological assays currently used for HIV screening.[11] The incidence of HIV infection among adults in the Middle East was estimated to be 0.3%, but increased by 20% during 2002.[9] In Saudi Arabia, the cumulative number of HIV-infected individuals was estimated at 1100 cases up to the year 2000, with an adult rate of 0.01%.[12] El-Hazmi[13] did not find any confirmed positive cases among 20423 Saudi blood samples. Madani et al[14] conducted an 18-year surveillance for HIV infections among Saudi citizens and found that the number of cases per 100,000 population varied widely between regions with a maximum of 74 cases, and a minimum of 2 cases. Badreddine et al,[15] analyzed 56 HIV-1 positive samples, and found that subtype C was the most common subtype present, and accounted for 39.3% of the infections followed by subtype G (25%), subtype B (17.9%), subtype D (3.6%), and subtypes A (1.8%), and CRF02-AG (1.8%). In addition, for 6 specimens subtype classifications were discordant between *gag, pol,* and/or *env*. Detection of HIV subtypes is important for epidemiological purposes, and may help in tracing the source of HIV infections. This study assesses the prevalence of HIV-1 genetic subtypes among infected Saudis, and compares these subtypes with others prevalent in different countries.

Methods. This is a retrospective study that was conducted in Dammam, Kingdom of Saudi Arabia, and in Abbott laboratories (USA) from 2004 to 2007. Blood samples were taken using standard techniques. Thirty-nine HIV-1 positive samples were included in the study. Patients included males (n=37), and females (n=2). The mean age was 34 years. No data were available about possible mode of transmission. The samples were serotyped using a research assay (MO2N assay, Abbott Laboratories, USA). Analysis for HIV-1 subtypes was carried out using the standard molecular techniques. This included reverse transcriptase-polymerase chain reaction (RT-PCR) amplification of segments of the viral genome (*gag* p24 and *env* gp41) followed by DNA sequencing and phylogenetic analysis. The phylogenetic analysis was performed using the PHYLIP software package (version 3.5c, J Felsenstein, University of Washington, Seattle, WA) with evolutionary distances estimated using Dnadist and phylogenetic relationships determined using Neighbor with SIVcpzGAB as the out group. The trees were constructed using TreeView (RDM Page, University of Glasgow, UK) and branch reproducibility was carried out using Seqboot (100 replicates) and Consense.[15-18] The obtained data from the HIV subtypes was compared with the data from other countries. Ethical approval of the Local Committee of Biomedical Ethics was obtained.

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Results. All samples were seropositive for HIV-1 group M. The M group was subclassified into 9 major clades, including A-D, F-H, J, and K, as well as several circulating recombinant forms. Of the 39 seropositive samples, only 12 were positive by PCR. Human immunodeficiency virus subtype C, the predominant strain, was identified among 7 (58%), while subtype B was found to be the second most common subtype; 2 (17%) of the 12 PCR-positive samples. Subtypes A (8%), D (8%) and G (8%) was detected in one sample. The phylogenetic tree was identified for the isolates (data not shown). In the phylogenetic tree, specimens are designated with subtype-sample ID.

Discussion. All samples were seropositive for HIV-1 group M thus, this finding is consistent with previous reports.[8,9] Among the M group, which accounts for over 90% of reported HIV/AIDS cases, viral genetic diversity is so great that this group has been subclassified into 9 major clades, including A-D, F-H, J, and K, as well as several circulating recombinant forms. Of the 39 seropositive samples, only 12 were PCR positive which is much lower than expected, and could not be due to antiviral therapy as none of the patients was on treatment. Instead, the use of serum that contains no preservative might have allowed virus degradation during freezing and thawing of the samples. Human immunodeficiency virus subtype C, the predominant strain, was identified, while subtype B was found to be the second most common subtype. These 2 subtypes represent 75% (9 of 12) of the samples, and is consistent with published data that showed subtype C, together with A, to be the predominant subtype in Asia.[9] Subtypes A (8%), D (8%) and G (8%) were

detected in one sample. Thus, a broad spectrum of HIV subtypes appears to be circulating in Saudi Arabia and may indicate various sources of acquiring the infection. Worldwide, the demographic distribution of patients infected with particular HIV-1 subtypes,[9] or subtypes are heterogeneous, with distinct regional distribution; A and A/G recombinant variants predominate in west and central Africa, whereas B the predominant subtype in Europe and the Americas. However, with increasing immigration and globalization, at least 25% of new infections in Europe are presently non-B African and Asian variants. Subtype B accounts for just above 12% of infections worldwide, whereas subtype C accounts for approximately 50%. Subtype C is largely predominant in southern and eastern Africa, India, and Nepal, and this subtype has created the recent epicenters of the HIV pandemic by its uncontrolled spread throughout Botswana, Zimbabwe, Malawi, Zambia, Namibia, Lesotho, South Africa, India, Nepal and China. Subtype D is generally limited to east and central Africa, with sporadic cases observed in southern and western Africa. The subtype E has never materialized alone, but rather appears as an A/E mosaic (CRF01-AE) detected in Thailand, the Philippines, China and Central Africa. Subtype F has been reported in central Africa, South America, and Eastern Europe. Subtype G and A/G recombinant viruses have been observed in western and eastern Africa as well as in central Europe. Subtype H has only been detected in central Africa. Subtype J has been reported exclusively in Central America. Subtype K has recently been identified in the Democratic Republic of Congo and Cameroon. Subtypes are genetically defined lineages that resolve through phylogenetic analysis as well-defined branches in a tree, the phylogenetic tree was identified for the isolates. In the phylogenetic tree, specimens are designated with subtype-sample ID (in other words, C-KFU10; subtype C-specimen KFU10). All were compared to reference strains representing all the major group M subtypes and CRF's. The values on the main branches are bootstrap values, and they indicate the percentage confidence in the branch; so for example, a value of 78 indicates that this branch is formed in 78 out of 100 trees evaluated. Horizontal branch length measures genetic distance. Phylogenetic analysis can help in establishing the origin of the circulating HIV subtypes, and may assess whether or not there are multiple viral introductions in the region.

Due to the limited number of samples used in our study, future studies should use more HIV-positive samples from different regions of Saudi Arabia. However, our data confirm, and adds to the recently published data on subtypes predominant in Saudi Arabia. The detection of HIV subtypes is important for epidemiological purposes, and for proper control of the HIV in the region.

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