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DETERMINATION OF HUMAN IMMUNODEFICIENCY VIRUS TYPE-1 AND ALLIED SUBTYPES IN SUDAN

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ABSTRACT

Introduction: Human Immunodeficiency Virus-1 (HIV-1) is the most common infection of an unresolved global disease that has had massive impact on human life since its emergence. Transmission of HIV-1 is still rapidly spreading despite identification over 33 years ago and an immense worldwide research effort to counter it.

Objectives: The aim of this study is to determine the HIV-1genotype and subtypes that cause AIDS in Sudan.

Methods: Samples were investigated and analyzed in three different laboratories; two in Sudan and the third one in Kenya, the Central Lab of Omdurman Military Hospital, Department of Microbiology, Virology Lab of Faculty of Medicine University of Khartoum and Kenya Medical Research Institute (KMERI) -Virus Research Center- Nairobi Kenya.

Results: HIV-1 was detected by RT-PCR at the virology lab and the result revealed (188) samples (90.9 %) positive for HIV-1 (12) samples (9.1%) were negative for HIV-1. Concerning HIV-1 subtypes or clades one hundred of EDTA samples were processed at Kenya Medical Research Institute (KMERI), Using hetero-duplex Mobility Assay Technique (HMA) for env gene. Three subtypes were detected: subtype (A) (46%), Subtype (C) (33%) and subtype (D) (21%). CD4 count was estimated before antiretroviral therapy and three month after treatment, it was found that 71% were responding and 29% were not.

Conclusion: The study concluded that the detected HIV subtypes in Sudan were subtypes (A) (C) and (D). Most of the patients were responding to ARV.

Key Words: AIDS, Genotype, Heteroduplex, Kenya, Serotype, Sudan

INTRODUCTION

Acquired Immunodeficiency Syndrome (AIDS) was first recognized as a new and distinct clinical entity in 1981. The first cases were recognized become of unusual clustering of diseases such as kaposi sarcoma and pneumocystis carinii, and pneumonia in young homosexual men, [1]. Analysis of HIV-1 genes of virus strains from different geographical locales has revealed that HIV-I can be divided into two distinctive groups, M (major) and O (outlier) [2]. HIV-1 group M isolates can be further subdivided into at least 10 distinct genetic subtypes (A-J) [3]. According to recent report by UN estimate in 2008 people living with HIV/AIDS is about 33 million 66.7% found in Africa [1]. HIV is an RNA virus from Lentivirus of the Retroviridae family [4] prevalent in Sudan with a figure of 1.6% among adults [5]. The first case reported was in 1986 and since then WHO become partner in the program [6]. Clinical presentation of HIV may usually pass through four phases depending on the status of immunity primary, early, intermediate and advanced HIV infection [7]. The presentation is ranging from reasonable mild to general (fever, urti, myalgia, arthralgia, lymphadenopathy, weight loss). Skin manfestaion, neurological (encephalopathy, headache, neuropathy), gastrointestinal tract manifestation (vomiting, diarrhea pharyngitis, oral ulcers, fungal infection), or

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respiratory system(like cough, shortness of breathing) [7], where WHO classification of the disease is according to the presentation rather than the immunological status[8]. AIDS cases were reported in other populations including intravenous drug users and hemophiliacs [9]. Blood transfusion recipients, adults from central Africa and infants born to mothers who themselves had AIDS or were intravenous drug users [9]. A virus related to human T-cell leukemia virus (HTLV-1) and (HTLV-2) was in criminated as a causative agent but later on the causative agent of AIDS was characterized and termed Human Immunodefiency virus Type-1 (HIV-1 and HIV-2) [10]. Sudan is a large country with opened boarders, and due to its geographical location, it is surrounded by nine countries some of which have a high incidence of HIV/AIDS. In addition, uncontrolled migrations of refigures across the borders, drought, famine in some areas, civil wars and difficult economic situations, resulted in immigration and displacement of many people whether from local population or from these neighboring countries. HIV is an important public health problem that prevalent at internationally, and nationally, Regional. The incidence of HIV/AIDS is on increase in Sudan and neighboring countries. The area of HIV diagnosis, management and monitoring are very important. Appearance of Resistance to antiretroviral therapy is increasing. Studies in the area of antiretroviral therapy and HIV drugs resistance are very essential. Such studies are very few in Sudan and are very strategic in the managements of HIV/AIDS [11,12]. The strains of HIV-1 can be classified in to three groups the "Major" group (M), the outlier group (O) and the New group (N). These three groups may represent three separate introductions of simian immunodeficiency virus in to humans; each type is divided in to subtypes and Circulatory Recombinant Forms (CRFs). Group (O) appears to be restricted to west and central Africa and Group (N), discovered in 1998, in Cameron, is extremely rare. More than 90% of HIV-1 infection belong to HIV-1 group (M) and unless specified the rest of this page relate to HIV-1 group (M) only. Within group (M) there are known to be at least nine genetically distinct sub types or (clades) of HIV-1. These are subtypes A, B, C, D, F, G, H, J, and K. Occasionally, two viruses of different sub types can meet in the cell of an infected person and mix together their genetic materials to create a new hybrid virus caprices similar to sexual reproduction and some new strain do not survive for long, but those that infect more than one person are known as circulatory recombinant forms (CRFs) [13]. The CRFs/A/B is a mixture of sub types A &B. The classification of HIV strain into subtypes and CRFs is a complex issue and the definitions are subject to some Subtypes into division of A1, A2, A3, F1 and F2 instead of A & F. Globally speaking, subtype A is the principal HIV-1 subtype found in Central and North African countries, sub type B is predominant in USA, Europe, Australia, Thailand and Brazil; subtype C is prevalent in south Africa, Ethiopia and India; CRFO_AE is common in south Africa. Information on HIV subtypes appears to divers in Iran, were HIV-1 subtypes A&B and has been reported among respectively intravenous by drug users and hemophiliac [14, 15].

MATERIALS AND METHODS

This cross-sectional study of HIV-1 was held to determine the seropositive patients from different areas of Sudan. Systematic random sampling were done by collecting blood, from South, North, West, East and Central Sudan. Forty samples were collected from 200 investigated patient's attending different clinics and hospitals in every area during two years of study period. The sample was a venous blood, collected in a plain and EDTA vaccutainer containers, from those suspected patient's positive for HIV-1. From those confirmed HIV-1 positive patient, 100 EDTA blood sample were chosen for the purpose of HIV-1 sub typing, the sub typing methodology was determined in Kenya Medical Research Institute (KMRI), Virus Research Center at the HIV Laboratory. Many techniques have been used to define subtypes of HIV-1 in Sudan, these include; serological test by ELISA, CD_4 count by FACS, Heteroduplex mobility assay (HMA) and subtype classification by polymerase chain reaction (RT-PCR). After processing the sample for sub typing, the amplified samples were run on an agarose gel 2% with ethiduium bromide for staining, and visualized .under UV light to confirm HIV-1. Amplicon was used to detect the subtype by specific subtype primer. The subtypes that detected by specific subtype for the universal groups M, N, and O; the Unipol5 for forward and Unipol6 for reverse direction (5'TGGGTACCAGCACA CAAAGGAATAGGAGGAA A3'and5'CCACAGCTGATCTCTGGCCTTCTCTGTAATAGA CC- 3'). For nesting PCR Universal for groups M, N, and O, the Unipol1 for forward and Unipol2 for reverse direction (5'-CCCCTATTCCTTCCCCTTCTTTTAAAA-3' and 5'-CCCCTATTCCTTCCC CTTCTTTTAAAA-3') [16], were confirmed by Heteroduplex Mobility Assay (HMA). After determining the HIV-1 subtypes, the patient given a dose of antiretroviral drugs which was Triomue (lamivudine, stavudine and Nevirapine) and after three months of treatment CD_4 count was estimated.

RESULTS

Determination of HIV- 1 genotype and subtypes is a key role in facilitating the treatment, trails of vaccination, and confirming the results of diagnosis. The Sudan, according to its geographical location is surrounded by countries with high HIV prevalence.

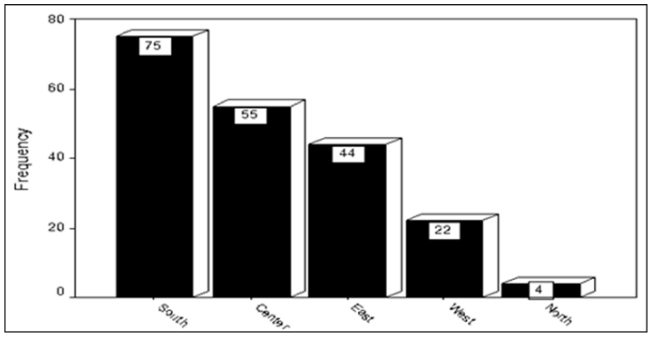

Figure 1: Geographical distribution of HIV including South Sudan State.

The Geographical distribution of the patients was, as follows: 75 patients from South of Sudan (37.5 %) 55 patients from the Center (27.5%) 44 patients from the East (22.5%) 22 patient from the West (11.5%) and 4 patients from the North (2.0%)

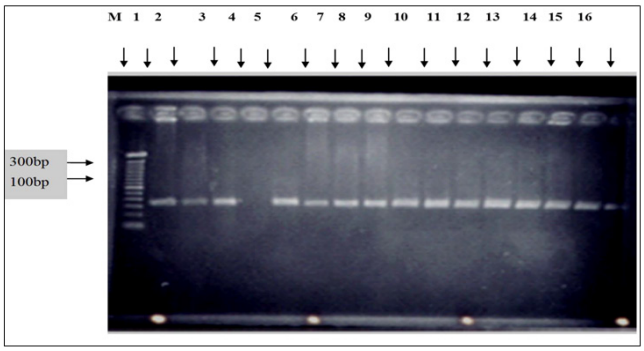

Figure 2: HIV -1 Positive samples ENV gene 100 -300 bp

One hundred – eighty eight (90,9%) were found to be HIV-1 positive samples ,twelve (9,1 %) were negative (-ve), pro-viral DNA in the DBS was amplified by nested PCR, and a 389-nucleotide segment of the C2-V3 env gene region was sequenced.

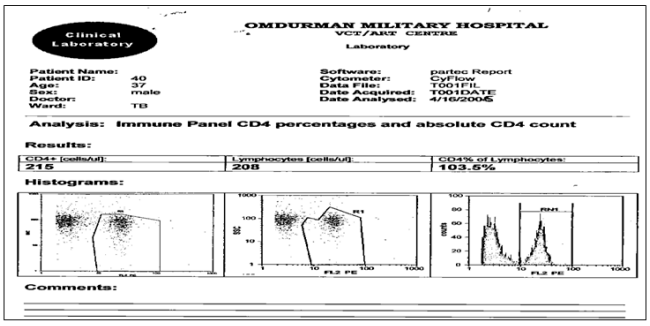

Figure 3: CD_4 count HIV (FACS) Partec Germany

Blood samples were analyzed on a Partect Automatic Machine to estimate CD¬4 count (FACS). The EDTA blood samples were divided in to two parts, one for the purpose of CD_4 count & the other for subtyping after extraction of provirus from peripheral mononuclear cells (PMNCs).

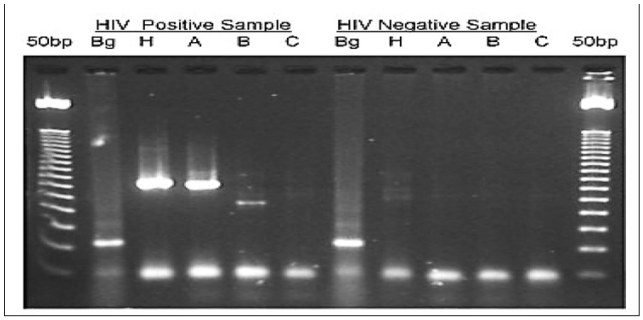

Figure 4: HIV -1 Subtype (A), (C) and (D)

After processing the sample for subtyping, the amplified samples were run on an agarose gel 2% with ethidium bromide for staining, and visualized .under UV light to confirm HIV-1. Amplicon was used to detect the subtype by specific subtype primer.

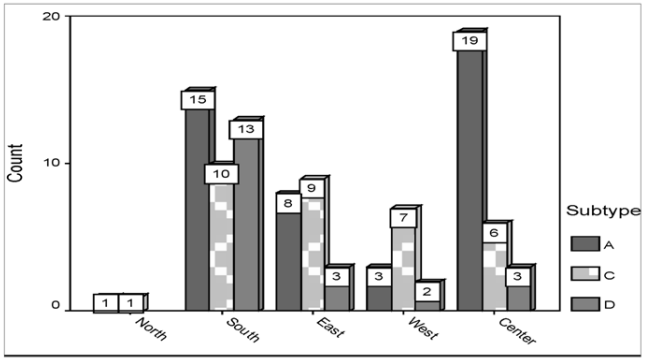

Figure 5: HIV -1 Subtype (A), (C) and (D) distribution in Sudan

The distribution of HIV-1 subtypes according to the different areas was; in North Sudan subtypes (A) n=1 (1.0%), subtype (C) n=1 (1%). In South Sudan subtype (A) n=15 (15%), subtype (C) n=10 (10%) and n=13 (13%) for subtype (D) .In East Sudan subtype (A) n=8 (8%), subtype (C) n=9 (9%) and subtype (D) n=3 (3%). In West Sudan subtype (A) n=3 (3%) subtype (C) n=7 (7%) and subtype (D) n=2 (2%).In Center Sudan subtype (A) n=19 (19%), subtypes (C) n=6 (6%) and subtype (D) was n=3 (3%).

DISCUSSION

Two hundred units of seropositive HIV Patients were tested by ELISA as reactive units then by using (RT-PCR) to detect HIV-1. For the patients' sex, 186 (84%) were male and 32 (16%) were female which is in agreement with HIV male to female ratio in Yemen that were 4:1 male to female [17,18], 88 (90.9%) were HIV-1 positive. The Geographical distribution of the HIV-1 in Sudan is closely related to the distribution of variants in neighboring countries [19]. Consequently, patients from South of Su-

dan before separation were n=75 (37.5 %), from Center Sudan n=55 (27.5%), from East Sudan n=44(22.5%), from West Sudan n=22 (11.5%) and from North Sudan (2.0%). The high prevalence estimated rate of HIV was found in South Sudan, and this was related to the geographical location of being near the high prevalence African countries [20]. However, this finding is disagreed with other authority where type A is the subtype that is common in west Africa but agree with them regarding subgroup (C) and (D) which usually found in central and east Africa(Sudan) [21,22]. However, to have subtype (A) dominant in Sudan is not strange since a good number of western Africa citizens migrate to Sudan throughout the last century to pilgrim. Determination of HIV-1 genotype and subtype is the key tools in determine the treatment, since a lot of gene mutation to the virus are reported [23]. Several techniques have been used to determine subtypes of HIV-1: The serological test by ELISA techniques, the CD_4 count by FACS, the Heteroduplex Mobility Assay (HMA) and subtype classification by polymerase chain reaction (RT-PCR).This assist the indication of the high specificity and sensitivity for HIV-1 subtypes. Compared with molecular diagnosis technique (RT-PCR) which was the golden standard (figure 2), the use of serologically defined subtype was mainly confined to subtype B infection among whites, as serotyping has been shown to be of good specificity for differentiating subtype B from non-B infections in populations predominantly infected with B subtype [24]. The result for HIV-1 sub typing were n=46 (46%) subtypes (A), n=33 (33%) subtype (C) and n=21 (21%) subtype (D), (figure 3). However, some studies suggest that the CD_4 count is a better predictor of disease progression than is plasma HIV-1 RNA in patients with very low CD_4 (counts >50 cells/mm^3) [25]. The Heteroduplex mobility assay (HMA) and multiregion hybridization assay (MHA) offered more affordable option for simple and rapid classification of HIV-1 subtypes in Sudan. However, due to cross-reactivity across subtypes, this method could not define specific sequence differences between isolates of the same or different HIV-1 subgroup (M). Therefore, the protocol was developed in combination with the (RT-PCR) as following; for subtype (A) by (HMA) and for subtype classification by polymerase chain reaction (PCR). The sequence analyses of envelope genes, using geographically diverse subtype reference sequences as well as envelope sequences of known subtype from Sudanese patients' genotyping. Because the RT-PCR genotyping system proved to be highly successful for the amplification of local strains with positive results [26], for the 100 cases a final algorithm of incorporating serology and genotypic subtype HIV-1 subtype was resulted as following; HIV-1 group (M) subtype (A) n=46 (46%), HIV-1 group (M) subtype (C) n=33 (33%) and HIV-1 group (M) subtype (D) n=21 patient (21%). The distribution of HIV-1 subtypes according to the different areas (fig 5) was agreed with local study of detecting HIV-1 subtypes in Sudan which were subtype (C) (30%) and subtype (D) (50%) [21], subtype (C) is similar to our result, but we were disagreed with that in the following that the commonest subtype we were detected was subtype (A). The transmission and distribution of HIV-1 subtypes is common and recognized as geographical distribution in the Democratic Republic of Congo which is closely related to the distribution of variants in neighboring countries the dominant strains are A, C and D. this would explain the difference in prevalence between the neighbor boarders [27]. In Sudan, subtype D is the most common [20]. However, its introduction here might have been from two fronts; from Central Africa and also from Uganda or Kenya where this subtype is prevalent the three subtypes detected, A, C, and D was shared with Kenya, Uganda and Ethiopia, and these countries became the main source of infection to Sudan [26, 27, 28]. Altlas et al [29] stated that; the HIV patients of African origin showed that 77% of them were responded to ARV triple therapy). The distribution of HIV-1 subtypes B9 and E in this study population is in line with several other studies into the molecular epidemiology of HIV-1 in Thailand.

CONCLUSION

Lastly we conclude that the detected HIV subtypes in Sudan were subtypes (A) (C) and (D). The high prevalence of HIV-1 infection in neighbor countries affected the citizens of Sudan's Border States with a significant impact of refugees' mobility across the country. The study showed that there was a significant association between CD_4 count and anti-retroviral therapy (ARV) the commonest subtypes that responding to dray were subtypes that responding to dray were subtype (A) and (D) the tubercles patients in subtype (C) treated with rifampicin were not responding to (ARVs).

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Informed Consent:

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Conflict of interest:

Since AIDS is a global disaster, the authors had decided to evaluate the disease situation in Sudan by using advanced protocols, techniques and analysis. All the authors who had participated in this work performed high calibrations in their contribution for establishing a bio-monitoring system for HIV-1.

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