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Rating of CCR5-Delta 32 Homozygous Mutation in Sudanese HIV Patients and Sex Workers

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Abstract

Background: Prevention against human immunodeficiency virus (HIV) includes natural resistance in the population; mainly frequency of cysteine-cysteine chemokine receptor type-5 (CCR5-delta 32 mutation). By knowing the frequency of this resistance in the community, the proportion of the population susceptible to infection can be determined. This study aimed to detect for the first time the rate of CCR5-delta 32 mutation in Sudanese individuals with HIV and sex workers. **Methods:** Cross-sectional study was followed in the parade from 2019 through 2021, study groups were Sudanese with HIV and sex workers. Sero-negativity of sex workers was confirmed by a rapid immunochromatography test (ICT). A blood sample was targeted for DNA isolation. PCR amplification was accomplished for CCR5 wild type and CCR5-delta 32 mutation genes using specific primers. **Result:** Among HIV patients, males, basic education level and ages below 60 years were commonly recorded while ages below 40 years, secondary education level and single marital status were predominated in sex workers. All HIV patients were positive for CCR5 wild type and negative for CCR5-delta 32 genotype. The sex workers group showed a frequency of 3.5% (97/200) for homozygous CCR5-delta 32 mutation. **Conclusion:** The rating of homozygous CCR5-delta 32 genotype in studied Sudanese sex workers was relatively more than other results obtained from African countries, and the mutation was significantly detected among sex workers group (P value = 0.008) when compared to the studied HIV group.

Keywords

CCR5-Delta 32, HIV, Sex Workers, Rating, Sudan

1. Introduction

According to the facts of the World Health Organization (WHO), human immunodeficiency virus (HIV) and AIDS is the most important threat to global health [1], despite this fact, the number of people living with HIV (PLHIV) is increasing rapidly [2]. The prevalence of HIV in Sudan increased in 1998 to reach 2% and remained at this level until 2020, as documented by the World Bank [3], this level keeps Sudan in the ranks of countries with a low prevalence rate, although its geographical surroundings with several highly affected countries. In 2016 estimated and reported number of PLHIV in Sudan was 56,000 and 21,471 respectively, while prevalence among sex workers increases to 1.3% [3].

One of the major co-receptor of HIV-1 and HIV-2 for entry into the human macrophages during initial infection is the cysteine-cysteine chemokine receptor type 5 (CCR5) [4]. Another important receptor necessary for HIV entrance is known as CXC chemokine receptor type 4 (CXCR4) [5]. Depending on the use of receptors CCR5 and CXCR4, the virus strain that uses receptors 5 is called R5-tropic, and studies indicated the predominance of this strain in the process of HIV transmission through sexual contact [6].

The most documented mutation of the CCR5 gene that affects HIV infection is known as the CCR5-delta 32 mutation; which could be existent as homozygous or heterozygous. In fact, people who are naturally protected against HIV carry homozygous type, while those who carry heterozygous have a late onset of AIDS symptoms [7]. Further to that, a study documented that individuals carrying homozygous type of CCR5-delta 32 gene remained uninfected with HIV-1 even with multiple exposures to the virus [8]. But in contrast, other findings concluded that protection against infection is related to both types of CCR5-delta 32 genotypes [9].

International surveillance of CCR5-delta 32 occurrence showed the highest frequency in North European countries, while the lowest rating was found in sub-Saharan countries [10]. Information regarding the existence and role of CCR5-delta 32 mutations in Sudan and most African countries is insufficient for a better understanding of the HIV transmission patterns [11]. On the other hand, the existence of the CCR5-delta 32 genetic mutations has negative effects that have been identified, represented in responses to chronic liver disease and antiviral drugs [12].

We observed only one published study in the Sudanese population done to identify the CCR5-delta 32 allele in patients with sickle cell anemia, in which the obtained result was none [12], thus the present study conducted to rate for the

first time the frequency of CCR5-delta 32 mutations in Sudanese HIV patients and sex workers.

2. Methods

Study Design and Setting

This cross-sectional laboratory based study was conducted at Wad Madani and Khartoum Antiretroviral Treatment Centers in 2019 and 2021. Wad Madani center is located in central Sudan in Gezira State which is a well populated area.

Selecting Participants and Variables

Participants of the study were adult volunteers randomly selected, and categorized into two groups; known patients with sero-positivity to HIV and healthy sex workers as a risk group. HIV patients were from Khartoum whereas sex workers were recruited from Wad Madani city in Gezira State. The HIV serological status of the sex workers group was negative. HIV patients participated before antiretroviral treatment (ART) administration. Variables of HIV patients and sex workers groups assessed in this study included socio-clinical and risk perception data.

Sampling

A total of 400 individuals were included; 200 from each group. Venous blood samples were obtained in EDTA anticoagulant. The formula of sample size is as the following:

The study sample size was 400 subjects which were determined according to the following formula:

$$n = \frac{z^2 pq}{d^2}$$

n: sample size, z: confidence level at 95% = 1.96, p: proportion in the target population to have a particular disease = 0.5, q: $1 - p$ and d: degree of accuracy desired = 0.05.

$$384 = \frac{1.96^2 * 0.5 * 0.5}{0.05^2}$$

DNA Extraction

The DNA was extracted using G-DEX IIb Genomic DNA Extraction kits; 300 µl of whole blood was added into 1.5 ml eppendorf tubes containing 900 µl RBC lysis solutions. Tubes were mixed thoroughly by vortexing and incubated for 5 minutes at room temperature and inverted again during the incubation. Centrifugation was done at 10.000 rpm for 1 minute. The supernatant was removed except for the white cell pellet and remained about 50 - 100 µl. The tube was vortexed vigorously and cells were resuspended. Then 300 µl cell lysis solution was added to the cells and shacked up and down to lyses the cells. A 1.5 µl RNase was added to the solution and incubated at 37˚C for 15 minutes. The sample was chilled at room temperature, and 100 µl of protein precipitation buffer was added to the cell lysate and vortexed vigorously at high speed for 20 seconds. Then a centrifugation at 13.000 rpm for 5 minutes was performed. The precipitated

proteins formed a tight white pellet. A 300 µl of supernatant containing the DNA was transferred into a 1.5 ml eppendorf tube. A 300 µl of 100% isopropanol was added and mixed by inverting gently for several times. The mixtures were centrifuged at 13.000 for 1 minute, the DNA was visible as a small white pellet. The supernatant was poured off and the tube drained briefly on clean absorbent paper. 1ml of 70% ethanol was added and the tube was inverted several times to wash the DNA pellet, and centrifuged at 13.000 rpm for 1 minute, then the ethanol was carefully poured off. Tubes were inverted and drained on the clean absorbent paper and allowed to air dry for 10 - 15 minutes. Finally, 150 µl of rehydration buffer was added and then rehydrate the DNA by incubating at 65˚C for 30 min. DNA was stored at −20˚C.

DNA purity was measured using a spectrophotometer at 260 nm wavelength and optical density ratio at the wavelength of 260/280 respectively.

Amplification of CCR5 Gene

PCR was done to amplify CCR5 wild type and CCR5-delta 32DNA using primer pairs (Macrogen, Europe, and Genomics). Primers were prepared as instructed by manufactures. 13 µl of nuclease free water and 1µl from each forward (5'CTGTGTTTGCGTCTCTCCCA'3) and revered primer (3'CCTCTTCTTCTCATTTCGACA'5) was added into PreMix tube Maxime and 5 µl from DNA to a final volume of 20 µl. Then the reaction was performed using a Bio-Rad thermocycler (USA), the program initiated with a first denaturation step at 94˚C for 5 min; followed by 30 cycles at 94˚C for 1 min, 65˚C for 1 min, 72˚C for 2 min, and a final extension at 72˚C for 5min and 4˚C hold temperature. Amplicon was resolved and screened using 1.5% agarose dissolved in 1XTBE (10.8 gm, Tris, 5.5 gm boric acid and 4 ml 0.5 M EDTA in a final volume of 1000 ml), and the mixture was boiled in a microwave. After cooling 5 µl of ethidium bromide (20 mg/ml) were added, mixed well and poured into a casting tray, and left to solidify at room temperature.

Data Analysis

The qualitative results of the study were analyzed descriptively for frequency determination. Chi-Square was calculated for association detection and the values less than 0.05 were considered significant. The Statistical Package for the Social Sciences (SPSS version 20) used analysis.

Ethical Approval

Ethical approval was obtained from the Gezira State Ministry of Health, Sudan and the Faculty of Medical Laboratory Sciences, University of Gezira, Sudan.

3. Results

For HIV participants, males accounted for 71% (142/200), the age group of 60 years and above were the less frequent at 5.5% (11/200) and basic education level was recorded at 88% (177/200). No co-morbidity or HIV treatments were documented (Table 1). In contrast, sex worker group involved participants in the secondary education level with percentage of 100% (200/200) and female rating reaching 54% (107/200) (Table 2). Yielded quality of extracted DNA of HIV

Table 1. Distribution of socio-demographic characters of Sudanese HIV participants. No 200.

		Frequency	%
Gender	**Male**	142	71
	Female	58	29
Age/year	**Less than 40**	97	48.5
	40 to 60	92	46
	More than 60	11	5.5
Residence	**Khartoum**	190	95
	Other	10	5
Occupation	**Students**	18	9
	Houswifes	35	17.5
	Drivers	11	5.5
	Workors	5	2.5
	Emoplyees	74	37
	Soliders	2	1
	Non-empolyee	55	27.5
Educational level	**University**	16	8
	Secondary	7	3.5
	Basic	177	88.5
Marital status	**Married**	94	47
	Single	105	52.5
	widowed	1	0.5
Co-morbidity	**Yes**	0	0
	No	200	100
HIV treatment	**Yes**	0	0
	No	200	100
HIV duration/year	**Less than 14**	0	0
	More than 14	200	100

Table 2. Demographics and risk factors observed among Sudanese sex worker participants. No 200.

		Frequency	%
Gender	**Male**	94	47
	Female	107	54
Age/year	**Less than 40**	121	60.5
	40 to 60	79	39.5
	More than 60	0	0

Continued

Residence	**Gezira State**	200	100
	Other	0	0
Educational level	**University**	0	0
	Secondary	200	100
	Basic	0	0
Marital status	**Married**	18	9
	Single	182	91
	widowed	0	0
HIV	**Yes**	0	0
	No	200	100
Sex frequency	**Daily**	0	0
	3 times/week	0	0
	Weekly	200	100
Condom use	**Often**	0	0
	Occasionally	200	100
	Rarely	0	0

viruses revealed an average ratio of 1.7 and a concentration of 105 μg/ml. The base pair for CCR5 wild and mutant types were 226 bp and 192 bp respectively (Figure 1). Wild type of CCR5 genotype was identified among all HIV patients subject and in 95.5% (193/200) of sex worker group while mutant CCR5-delta 32 genotype was detected only in 3.5% (7/200) of sex workers. A Chi-Square value of 0.008 was found as a significant association of CCR5-delta 32 genotype detection between HIV and sex workers groups (Table 3).

4. Discussion

Genetic studies related to HIV/AIDS in the population help to understand susceptibility to the disease and opportunities for control, treatment and vaccination [13]. Throughout the world, one of the most documented natural resistance genetic characters against HIV acquisition is the presence of CCR5-delta 332 mutation, however, many countries such as Sudan lack the necessary information [10]. This is almost due to reasons related to the available resources that limit the implementation of such projects, especially the cost of national surveys, thus up to date, no findings were recorded about the frequency of the CCR5-delta 32 gene among the Sudanese population. Some conclusions have also limited the existence of CCR5-delta 32 mutation in the regions of North Europe, West Asia and North Africa [14], such a study indicates the possibility of having a mutation in the Sudanese population.

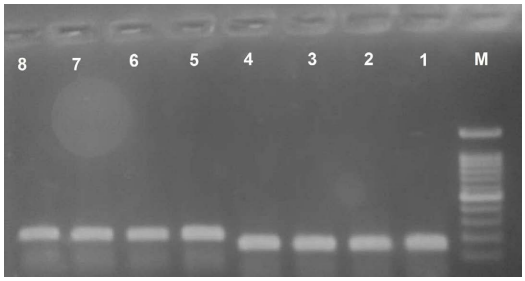

Figure 1. Agarose electrophoresis of isolated DNA from HIV patients and sex workers. Lane 1, 2, 3 and 4 were 192 bp of CCR5-delta 32 type. Lane 5, 6, 7 and 8 were 226 bp of CCR5 wild type. Lane M 100 bp DNA ladder.

Table 3. Distribution of CCR5 genotypes (homozygous and heterozygous) among HIV and sex workers groups.

Study subject	CCR5-delta 32		Total	P. value
	CCR5/CCR5	CCR5-delta 32/CCR5-delta 32		
HIV cases	200	0.0	200	
Sex workers	193	7.0	200	0.008
Total	393	7.0	400	

Results of this study indicated what had been stated globally; HIV in Sudan mostly affects those ages that are less than 50 years [3]. The records of the study of HIV cases showed that males were more infected in Sudan; the reason for this is the effectiveness of the transmission of HIIV through sex among men, which had been confirmed in a previous study [15]. The current study observed an accumulation of HIV cases at a low educational level; this is an epidemiological indicator that eliminates sufficient knowledge of transmission methods of HIV infection and prevention measures [16]. On the other hand, the sex workers group of this study also lacks a high level of education which could be linked to low personal income and instability.

From all existent data regarding the presence and frequency of CCR5-delta 32, it is difficult to say whether it is only associated with a particular race or not. And it can be said that the rating of presence varies, as the countries of Northern Europe have high rates of presence and East Asian [17] and Africa have the lowest [18]. Information about rating of CCR5-delta 32 genetic mutation in Africa is very limited and insufficient to be generalized, on the other hand, the hypothesis that there is resistance to AIDS in some African populations has been studied as indicating a resistance relationship between the CCR5-delta 24 mutation that has an effectible impact on the CCR5 addressing [19].

Our result is the first finding of the homozygous CCR5-delta 32 gene among Sudanese with HIV and sex workers; it is a higher recorded rate than in most African countries, which could be referred to as the characteristic of the Sudanese human being, in which black and white elements are mixed [20] [21]. In line, our result is not in agreement with other studies conducted on the African

populations from Nigeria [22] and Cameroon [23]. The result yielded from this study, indicated that the rating of CCR5-Delta 32 Homozygous mutation was 3.5% in Sudaneses HIV patients and Sex Workers, while in some studies in African population indicated the absence of this mutation, their results may differ when studying a larger sample size representing the whole community. Since the homozygous gene is inherited from two carrier parents, the obtained percentage of the CCR5-delta 32 allele indicates the presence of more carriers in the Sudanese population. The conclusion of this study is the presence of the gene responsible for HIV resistance in sex workers in a significant proportion compared to the HIV studied patients. Also, the studied Sudanese subjects showed a higher percentage of the gene than most African countries, and within the limits of what is had been estimated for African countries north of the Sahara.

Study Limitation

This study was limited to two studied groups that do not represent the entire Sudanese population, which consists of several different races.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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