

Descriptive Serological Diagnostic Techniques of HIV and AIDS Infections Amongst Adults Persons in Maiduguri, Nigeria

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Abstract: This research study was conducted on the Descriptive Serological Diagnostic Techniques of Human Immunodeficiency Virus and Acquired Immune Deficiency Syndrome (HIV/ AIDS) infections amongst adult persons in Maiduguri, Nigeria. 108 blood samples of the volunteers were randomly collected and analysed; 52% (56) are males, while 48% (52) are females. Serological and CD4 cells count techniques were applied. 45 persons were infected, had a total of 42% Seropositivity of infections with HIV/AIDS. Out of the total Seropositive, 64% (29) are females and 16 36% (16) are males. The results revealed that, less than 20 years old are 20% (9), 21 to 30 years are 38% (17), 31 to 40 years are 36% (16), 41 to 50 years are 7% (3) and lastly, those within age range of 51 to 60 years old were found to be seronegative. CD4 count revealed that, those showed seropositive of the total percentage prevalence of HIV/AIDS infection within the range order of the CD4 count range (200/ μ l), are 4% and are at risk of weaker immunity or immunodeficiency. Conclusively, those within the age of 20 to 40 years are sexually active, are at risk factor and the females had the highest seroprevalence. Variables obtained are subjected to simple statistical tools; percentage, mean and standard deviation. The results obtained in this study are accepted and support the works of most authors, the Government and Non-Governmental Organizations should create awareness and positive perceptions on the malignant disease, HIV / AIDS in the study area, Nigeria and the entire world at large.

Keywords: HIV / AIDS, HIV-1 and HIV-2, CD4 Count, Seropositivity, Seroprevalence, Serological Diagnosis

1. Introduction

Acquired Immuno Deficiency Syndrome (AIDS) is caused by the Human Immunodeficiency Virus, (HIV), belonging to the family retroviridae and subfamily lentiviridae. The disease is known as “*Kanjamau*” in Hausa language, meaning serious and continuous emaciation of the casualty, i.e. infected person. During infection with HIV, CD4 + T-Lymphocytes, as well as other cells of Immune system are infected and depleted leaving the patient progressively immune compromise [4, 14]. As the CD4 + T-Lymphocytes

decreases, patients are at inflammatory condition and malignancies [14]. The long incubation period of HIV infection and development of AIDS ensures that the surveillance of HIV infection always exceeds the number of AID cases [10, 12]. In the early 1980, unusual occurrence of Pneumoniosis Carnii pneumonia and seromia in previously healthy young men in USA lead to the recognition of AIDS as anew disease syndrome [2, 3, 4, 12, 13]. An investigative team led by Montagner at Paster Institute in Paris and Gallo at National Institute of Health under took studies to isolate and identifies Retrovirus from patient with AIDS pre-AID condition in 1983 – 1984 [14, 17]. The human Retrovirus

was isolated from the T- cell of patient with T- cell leukemia or human T- lymphotropic Virus type -1 or HTLV- 1. Since then the case of the acquired immune deficiency syndrome (AIDS) has been shown to be a Retroviruses, also prediction for T- cell deferring significantly from HTLV-1 [5, 9 16]. In sub-Sahara Africa, HIV is mainly transmitted through sexually and prenatal. HIV-1 correlates with the risk of sexually transmission [12, 9, 20]. CD4+T-lymphocytes depleted Vitamin A deficiency; certain sexually transmitted infection (STI) and pregnancy are associated with genital shedding of HIV 1. HIV infections give rise to whole array of clinical problem which alter a variable period of time will climate in full blown of AIDS and then death [2, 8, 10, 16, 19]. HIV infection is chronic medical condition, which involves all system in the body. HIV has a various clinical stages. These include; Short primary infection, advance disease (AIDS), and prolong as systematic stage. The advance disease AIDS characterized by severe depression of cellular immunity (CD4 cells count below 200/ μ l) and both infectious and non-infectious complication referred to opportunistic illnesses [2, 8, 9]. HIV infection causes a progression of symptom and signs at first where are none, and then a seroconversion reaction may occur [4,8,14]. Primary HIV infections (acute HIV infections) are presents in the initial viremia when the antibodies are developing. This acute manifestation is usually seen in about 50 to 90% of patient [2, 23]. The first case of AIDS in Nigeria was in 1984, a sexually active 13 years old girl, but reported two years later in 1986, since then the National control programmed has been in place [12, 13]. In Nigeria, pregnant women in cities are HIV positive to HIV-1. HIV-2 is also endemic in West Africa, however, the province has been relatively low [13]. Hence, there is the need to carry out a periodic retrospective and prospective survey, in order to determine the epidemiological status of HIV in the Maiduguri, the North-Eastern part of Nigeria. The objectives of the study are to investigate the prevalence of HIV- AIDS Virus infection in this area and to determine the possibility of HIV as single most important cause of viral infection of both women and men.

2. Methodology

2.1. Study Area and Location

The study was conducted in Maiduguri, Nigeria. It has an area land-mark of 300 square kilometres (300 km²), which lies between latitude 12^o North to 13^o North and longitude 13^o East to 15^o East respectively. The climatic condition is of a hot dry season (27^oC to 42^oC), and an annual rainfall of 500 to 600 mm has been recorded [6]. It has an estimated population of 629,486 people, out of which 340,809 are males and 288,977 are females [11].

2.2. Materials

2.2.1. Reagents Used

0.05% solution of sodium hypochlorite, 0.09% sodium azide

solution, 70% alcohol solution, Buffer phosphate saline, Tap water, Wash buffer, Western blot reagent, CD4+ count reagent, PARTEC GmbH no Lyse buffer, CD4 mAb PE (MEM-241 PE-conjugated monoclonal antibody two human CD4).

2.2.2. Apparatus Used

Micro pipettes, Micro pipette tips, Disposable hands gloves, Mouth mask, Laboratory coat, Wastes bin, Western blot kit (Immunetics –Qualicode™ HIV – 1 / 2 kit), Cotton wool, PARTEC flow cytometric Instrument/Device, Partec test tubes, EDTA bottles, Forceps (blunt), 5ml Syringes / Needles (Sizes: 38 × 0.8mm), Tourniquet, Time watch (stop watch), Rocking flat form, Vacuum system (water aspirator with trap), Graduated cylinders (25ml and 1L capacity), Automatic pipette for dispensing volumes of 10 μ l to 1ml, Flask (25ml and 1L), Multichannel pipette.

2.3. Methods

2.3.1. Blood Samples Collection

After disinfecting the top of the table and the area with 70% alcohol swab and then later with the solution of sodium hypochlorite swab, all the materials required for the blood samples collection were placed on the table ready for to be used. The tourniquet was tied on to one of the upper arm of the patient for prominent vein to be appeared (veni-puncture was been applied). 70% alcohol was swabbed on the venous area in order to disinfect the area where the needle of the syringe to be inserted. The veni-puncture was performed by collecting 5ml of whole blood samples and was dispensed in to the EDTA bottle, tilted, well-mixed in order to prevent the blood from clotting. All the bio-data (age, sex, ward, occupation, etc.) were collected, recorded, and sample identification number were given and written on the each sample bottle respectively. Samples were collected aseptically, properly arranged and ready for the analyses as stated in this study research.

2.3.2. Samples Analyses

The blood samples collected were subjected to the following techniques for the analyses and the detection of HIV/AIDS antibodies. These are;

1. The PARTEC CD4+ count for the identification and the enumeration of the CD4+ helper/inducer T-lymphocytes subset for the studying of HIV, as described by PARTEC easy count kit by Partech Healthcare Immunology, (2009).
2. Detection of antibodies of any immunoglobulin class specific to the recombinant HIV-1 and HIV-2 as described by Trinity Biotech Uni-Gold™, (2005).
3. Detection of human immunoglobulin recombinant HIV specifically by using Western Blot techniques as described by Immunetics – Qualicode™ HIV – 1 / 2, (2007).

(i) CD4+ Helper/Inducer T- Lymphocytes Count

(a) Principles

Approximately, 20% to 60% of human peripheral blood mononuclear cells as well as a subpopulation of monocytes

but with a weaker signal are stained. The mouse monoclonal antibody MEM 241 recognises the human CD4 antigen, a transmembrane glycoprotein (55 kDal) of the immunoglobulin supergene family, present on a subset of T-lymphocytes ('helper/inducer' T-cells) and also expressed at a lower level on monocytes, tissue macrophages and granulocytes [15].

(b) Procedure

20µl of well-mixed whole blood (EDTA as an anticoagulant used) was added to a Partec test tube. 20µl of CD4 mAb PE mixed gently and incubated for 15 minutes at room temperature and protected from light. 800µl of no lyse buffer was added, shake or vortex gently. The blood sample preparation was analysed by using Partec device (Partec flow Cytometre) with an excitation light sources of 488nm or 532nm (blue or green solid state laser) and counting was carried out. All procedures were observed, carried out correctly and aseptically for an optimal performance as described by PARTEC GmbH, (2009).

(c) Safety Precaution Taken

Reagents were stored at -8°C in the dark. All samples and reagents were handled with care and brought to room temperature before been utilized. Working area was disinfected; the laboratory bench was cleaned and disinfected with 70% alcohol and 0.05% sodium hypochlorite before and after the daily work. All standard laboratory bio-safety regulations were observed. Protective wears are worn. Hands are washed before and after the laboratory work daily.

(ii) Trinity Biotech Uni-Gold Techniques for Detecting HIV

(a) Principles

Recombinant proteins representing the Immuno dominant regions of the envelope proteins of HIV-1 and HIV-2, glycoprotein gp41, gp120 (HIV-1) and glycoprotein gp36 (HIV-2) respectively are immobilized at the test region of the nitrocellulose strip. These proteins are also linked to colloidal gold and impregnated below the test region of the device. A narrow band of the nitrocellulose membrane is also sensitized as a control region. During testing drops of Serum, Plasma or whole blood is applied to the sample port, followed by two drops of wash buffer and allowed to react. Antibodies of any immunoglobulin class specific to the recombinant HIV-1 or HIV-2 proteins, will reach with the colloidal gold linked antigens. The antibody protein colloidal gold complex moves chromatographically along the membrane to the test and control regions of the test device. A positive reaction is visualized by a pink/red band in the test region of the device. A negative reaction occurs in the absence of human immunoglobulin antibodies to HIV in the analyzed specimen. Consequently no visually detectable band develops in the test region of the device. Excess conjugate forms a second pink/red band in the control region of the device. The appearance of this band indicates proper performance of the reagents in the kit [22].

(b) Procedures

Reagents and samples are removed from the refrigerator, placed on the bench and allowed to stand for 20 minutes to reach room temperature. The required numbers of the Trinity Biotech Uni-Gold HIV test device are removed from their protective wrapper. By using the disposable pipettes supplied, it was filled with the well-mixed whole blood sample; two drops were carefully dispensed into the sample port (approximately 60µl). Two drops (approximately 60µl) of the wash reagent was added in to the sample port carefully. It was then allowed to stand for 10 minutes from the time of addition of wash reagent for reaction to be occurred. The results were read (after the 10 minutes incubation time) and recorded immediately. All procedures were observed, carried out correctly and aseptically for optimum performances as described by Trinity Biotech Uni-Gold HIV test, (2005).

(c) Safety Precautions Taken

Results are taken and recorded immediately after 10 minutes of incubation period for the reaction to be occurred completely. Reagents were stored at -8°C in the dark. All samples and reagents were handled with care and brought to room temperature before been utilized. Working area was disinfected with 0.05% solution of sodium hypochlorite and 70% alcohol. All standard laboratory bio-safety regulations were observed. Protective wears are worn. Hands are washed before and after the laboratory work daily.

(iii) Western Blot Test (Immunoassay Techniques) for HIV Antibodies Detection by Using Qualicode™ HIV – 1 / 2 Kit

(a) Principle

The test detects lower level of HIV antibodies within three months of infection by HIV – viral particle. In this test a blood samples is applied on to a paper strip containing HIV proteins, producing a colour change on the paper [5]. The Qualicode™ HIV – 1 / 2 kit is a qualitative Immunoblot assay based on the Western blotting principle. The assay is performed on an Immunoblot membrane containing HIV – 1 viral lysate proteins (HTLV-111B strain) and a recombinant HIV – 2 proteins. To produce the membrane, HIV – 1 viral lysate proteins are fractionated according to molecular weight by electrophoresis on a polyacrylamite slab gel (PAGE) in the presence of sodium dodecyl sulphate (SDS). The separated HIV – 1 polypeptides are then transferred via electrophoretic blotting from the gel to a nitrocellulose membrane. Two bands are directly stripped on the membrane, a control band comprising Staphylococcus protein A and a recombinant HIV-2 specific envelope antigen. The membrane is then cut into strips for individual sample testing. During the procedure, the strips containing the HIV -1 / 2 protein are reacted with serum specimens and washed to remove unbound antibodies. Visualisation of human immunoglobulins specifically bound to HIV -1 or HIV-2 proteins is performing by sequential reaction with goat anti-human immunoglobulin-alkaline phosphate conjugate and BCIP / NST substrate. Band position are compared to those

on the Reference card developed using the HIV-1 / 2 positive control serum. The intensity of the bands is monitored by comparison to the HIV-1 / 2 weakly Reactive controls [18].

(b) Procedures

These are from the strips preparation, sample addition, conjugate addition and up to substrate additions were carefully observed and quality control was assured. The assay procedures are followed step by step and all reagents are prepared as described in the package insert of the kit provided. The kit components was stored at -8°C, removed and was allowed to reach room temperature before had used. Human sera were used as sample specimens. Results were taken by interpretation, that is, tests are compared with Reference Card.

(c) Precaution

The expiration time of the kit components was noted and found ok for the immunoassay procedures. Non haemolysed blood samples were used for the analyses. Bio-safety level two (2) of the Bio-safety in Microbiological and Biomedical Laboratories were observed carefully.

2.4. Data Analysis Used

Data obtained from this research study were subjected to statistical analysis by using means for the measurement of central tendency, and standard deviations for measurement of dispersion and or discrepancy within the variables (determined in triplicates) reported in percentage as described by Stroud and Booth, (2001) and as performed by Gwana *et. al.*, (20140).

3. Results

The results of the study in Table 1 showed the number of person involved in the study, 56 (52%) are males while the females are 52 (48%), and that of Table 2 showed the total number screened for HIV/AIDS, with seroprevalence of 45 (42%) adult persons were found to be infected, showed seropositive; females with 29 (64%), while the males are with 16 (36%).

Table 3 showed the numbers of the persons screened for HIV/AIDS according to their age-group distribution; those that are less than 20 years old are 15 (14%), 21 – 30 years are 47 (44%), 31 – 40 years are 35 (32%), 41 – 50 years old are 10 (9%) and lastly 51 – 60 years old are 1 (1%).

Table 4 showed those who were found to be seropositive on the analyses of their blood samples, according to their age – group distribution are; less than 20 years old are 9 (20%), 21 – 30 years are 17 (38%), 31 – 40 years are 16 (36%), 41 – 50 years are 3 (7%) and lastly those that are within the age – range of 51 – 60 years old are found to be none (0%).

Table 5 showed those males who were seropositive on the screening of their blood samples and according to their age – group distribution are; less than 20 years old were not found (0%), 21 – 30 years are 6 (38%), 31 – 40 years are 8 (50%), 41 – 50 years old were found to be 2 (13%) and while those within the age – group of 51 – 60 years are none (0%).

Table 6 showed the age – group distribution of the females that were found to be seropositive on the bases of the analyses of their blood samples are; those that are less than 20 years are found to be 9 (31%), 21 – 30 years are 11 (38%), 31 – 40 years are 8 (28%), 41 – 50 years old was 1 (3%) and those within the age – group of 51 – 60 years was found to be none (0%).

Table 7 showed the cells count (the CD4 – T- lymphocytes count) of the whole blood samples that are found to be seropositive on immunoassay analyses when screened for HIV/AIDS; less than 200 cells per microlitre (cells/μl) of whole blood are 2 (4%), 201 – 300 cells/μl are 5 (11%), 301 – 400 cells/μl are 18 (40%), 401 – 500 cells/μl are 8 (18%), 501 – 600 cells/μl are 5 (11%), 601 – 700 cells/μl are 3 (7%), 701 – 800 cells/μl are none (0%), 801 – 900 cells/μl are 2 (4%) and lastly those within 901 – 1000 cells/μl were found to be 2 (4%).

Table 1. Number of Persons Screened for HIV According to their Sexes

Serial number.	Sex.	Number of persons involved.	Percentage
1.	males	56	51.85
2.	females	52	48.15
Total (Mean ± SD).		108 (54 ± 2).	100 (50 ± 1.9).

Table 2. Number of Prevalence of HIV Diagnosed According to their Sexes.

Serial Number.	Sex.	Number of prevalence.	Percentage
1.	Males	16	35.56
2.	Females	29	64.44
Total (Mean± SD).		45 (22.5 ± 6.5).	100 (50 ± 14.4).

Table 3. Number of Persons Screened for HIV According to their Age-Group Distribution.

Serial Number.	Age- Group.	Number of persons screened	Percentage. (Years).	For HIV.
1.	< 20	15		13.88
2.	21 - 30	47		43.52
3.	31 – 40	35		32.41
4.	41 – 50	10		9.26
5.	51 – 60	1		0.93
Total (Mean± SD).		108 (21.6 ± 16.9).		100 (20 ± 15.6).

Table 4. Number of Prevalence of HIV Amongst the Persons Screened According to their Age – Group Distribution.

Serial Number.	Age - Group.	Number of Positive Percentage. (Years).	Cases of HIV.
1.	< 20	9	20
2.	21 - 30	17	37.78
3.	31 – 40	16	35.56
4.	41 – 50	3	6.67
5.	51 – 60	0	0
Total (Mean± SD).		45 (9 ± 6.8).	100 (20 ± 15).

Table 5. Prevalence of HIV Amongst the Males Screened According to their Age- Group.

Serial Number.	Age-group (years).	Positive cases of HIV.	Percentage.
1.	< 20	0	0
2.	21 - 30	6	37.5
3.	31 – 40	8	50.0
4.	41 – 50	2	12.5
5.	51 – 60	0	0
Total (Mean± SD).		16 (3 ± 3).	100 (20 ± 20).

Table 6. Prevalence of HIV Amongst the Females Screened Accounting to their Age-Group Distribution.

Serial Number.	Age-group (years).	Positive cases of HIV.	Percentage.
1.	< 20	9	31.03
2.	21 - 30	11	37.93
3.	31 – 40	8	27.59
4.	41 – 50	1	3.45
5.	51 – 60	0	0
Total (Mean± SD).		29 (5.8 ± 4.4).	100 (20 ± 15.3).

Table 7. CD4 Count of the Adult Persons that are Seropositive on the analyses of the blood samples according their range order

Serial Number.	CD4 Count Range.	Prevalence.	Percentage. (Cells /1µl of blood).
1.	< 200	2	4.44
2.	201 - 300	5	11.11
3.	301 - 400	18	40.00
4.	401 – 500	8	17.78
5.	501 - 600	5	11.11
6.	601 – 700	3	6.67
7.	701 – 800	0	0
8.	801 - 900	2	4.44
9.	901 – 1000	2	4.44
Total (Mean± SD).		45 (5. ± 5).	100 (11 ± 11).

KEYS: SD -- means Standard Deviation

/ -- means per

1µl -- means one microlitre

4. Discussion

The prevalence of HIV/AIDS amongst adult persons in Maiduguri Metropolitan Council of Borno state, north – eastern Nigeria, was carried out on one hundred and eight (108) blood samples, collected from adult persons who were selected randomly. Serological and CD4 count analyses had been done which led to these results that had been obtained. Out of the stated number of the adult persons screened, 56 were males and 52 were females. A total of 45 adult persons were found to be seropositive, i.e. are infected with HIV/AIDS, while the remaining number of the persons (64 adult persons) are seronegative, i.e. having not infected with the malignant disease, HIV/AIDS.

Amongst those that are seropositive, 16 persons are males, while the remaining numbers of persons (29) are females respectively. Thus, the total infection of HIV/AIDS showed a prevalence of 42% out of the total numbers of the 108 adult persons screened. The females are having the highest with 64%, while the males are with 36% respectively. It was observed that, the numbers of persons screened for HIV/AIDS with accordance to their age – group distribution are; those within the age – group of 21 – 30 years old appeared to be the highest with 44%, followed by 31 – 40 years old with 32%, less than 20 years old are with 14%, then 41 – 50 are with 9% and the least are those within the age – group of 51 – 60 years old are with 1% respectively.

With regard to the number of prevalence of HIV/AIDS

amongst the adult persons screened in accordance to their age – group distribution are; 21 – 30 years old are the highest with a prevalence of 38%, then followed by 31 – 40 years old are with 36%, less than 20 years old are having 20%, 41 – 50 years old are with 7% and the least are those that fall within the age – group distribution of 51 – 60 years old with zero percentage. The results shows that, 21 – 30 years old are considered to be sexually active and exposed to the risk factor. 31 – 40 years old and those within less than 20 years old (Teenagers or adolescences) who are also support to be sexually active or reached puberty age are probably exposed to the risk factors.

Also found out in this research study, the number of prevalence of HIV/AIDS among the males that had been screened according to their age – group distribution are; those having the highest are 31 – 40 years old with 50%, then within the age – group of 21 – 30 years old are with 38% and the least are those within 41 – 50 years old with 13%. This means that, the highest in these groups are those within the age – group of 31 – 40 years old amongst the males, probably is due to sexually active condition of the group that were exposed. In another development, the results obtained showed that the number of prevalence of HIV/AIDS amongst the females screened in according to their age – group distribution are; the highest are those within the age – group of 21 – 30 years old with 38%, then followed by those less than 20 years old are with 31% and those within the age - - group of 31 – 40 years old are with 28%, and the least are those that fall within the age – group of 41 – 50 years old are with 3% only. Thus, it is probably due to ageing and sexually active with exposure to the risk factors.

Apart from the serological analyses, of which Immunoassay was carried out, a cells count of the CD4 count had been conducted on among those that showed seropositive of the total prevalence of 42% out of the 108 adult persons screened for HIV/AIDS. The results obtained showed the percentage prevalence of HIV/AIDS infection within the range order of the CD4 count range. The highest are those who fall within the cells – count range of 301 – 400 cells per microlitre of whole blood samples with 40%, then followed by those within the cells – count range of 401 – 500 cells/ μ l are with 18%, those within the range of 201 – 300 cells/ μ l and 501 – 600 cells/ μ l are having 11% each respectively. Those within the cells count range of 601 – 700 cells/ μ l of whole blood sample are with 7%, then those within the cells count range of less than 200 cells / μ l, 801 – 900 cells/ μ l and 901 – 1000 cells/ μ l were with 4% each respectively.

The normal range or safer cells count of the CD4 count is 1000 cells per microlitre and above of whole blood, while the cells count range that is at danger or risk factors is less than 200 cells/ μ l. The results, in this study showed that, only 4% are at risk of weaker immunity or immunodeficiencies, i.e. only two (2) adult persons are found to be at risk factor of weaker immunity stage. In conclusion, it was observed in the study that, all the variables obtained are subjected to simple statistical tools; percentage, mean, standard deviation and the results obtained are that, the standard deviation is

less than or equal to the mean. That means the results obtained in this research study are accepted.

5. Conclusion and Recommendations

5.1. Conclusion

The prevalence of HIV/AIDS amongst adult persons in Maiduguri, North – eastern Nigeria, was carried out on one hundred and eight (108) blood samples, collected from adult persons who are selected randomly. The results obtained are based on the serological and cells count of the CD4 count analyses, using the Uni-Gold™ and Western Blotting - Qualicode™ principles are strictly observed and the CD4 Count was performed by using PARTEC CD4 easy count kit. Out of the total number screened for HIV/AIDS, 45 (42%) adult persons were found to be infected with disease and showed seropositive, females with 29 (64%), while the males are with 16 (36%). The Cells count of the CD4 count had been conducted on among those that showed seropositive of the total prevalence of 42% out of the 108 adult persons screened for HIV/AIDS. The results obtained showed the percentage prevalence of HIV/AIDS infection within the range order of the CD4 count range. The normal range or safer cells count of the CD4 count is 1000 cells per microlitre and above of whole blood, while the cells count range that is at danger or risk factors is less than 200 cells/ μ l. The results, in this study showed that, only 4% are at risk of weaker immunity or immunodeficiency, i.e. only two (2) adult persons are found to be at risk factor of weaker immunity stage. The variables obtained from this study are found significant and are accepted.

5.2. Recommendations

We recommended that, both the Government and Non-Governmental Organizations should create awareness and positive perceptions on the malignant disease, HIV / AIDS in the study area and Nigeria at large. This should be done through locally and nationally, by involving the youths in the enlightens programmed at all levels of education, both traditional and modern educations, traditional rulers, chiefs, religion leaders and tradition healers on the danger and prevention of the disease conditions and it causative agents at both vertical and horizontal stages.

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