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Detection of HIV p24 antigens, CD4/CD8 counts and HIV RNA quantification among HIV patients, at hi-tech center, in cosmopolitan city of Chennai, Tamil Nadu, India

Amirthalingam R[1☼], Pavalakodi VN[2]

1. Molecular Biologist, Dept. of Mol. Bio., Hi-Tech Center, Chennai, T.Nadu, India
2. Senior Lecturer, Dept. Biochemist, Apollo Science College, Chennai, T.Nadu, India

☼***Correspondence:*** Specialist, Department of Molecular biology, Hi-Tech Diagnostic Center, Chennai, T.Nadu, India. P.O.Box. 600017- email:amrithrgenes@yahoo.co.in

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ABSTRACT

A total of 349 suspected cases were included in the study with 50 controls. Off these, there are 76 females (21.77%) and 273 males (78.23%) of HIV suspected cases with different age groups(19-60) have been collected, processed with quality controls and incorporated in present study, during the years of 2010-2012 in hi-tech diagnostic center, Chennai, Tamil Nadu India. All patients have been screened for routine, HIVp24 antigen, CD4&CD8 counting and HIV RNA quantification. The FDA approved medical devices have been applied for above diagnostic tests likes Sysmex 2000i, Roche Cobas Integra 400plus, FACS caliber system and Abbott2000rt respectively. High risk age groups (25-40 years) have been observed in males and females suspected cases and it were higher in males (71.26%) when correlative with females infected cases (21.4%). The actual viral load quantification were obtained as results in different ranges like 14.30% target not detected(TND),7.7%(< 40 copies/ml),5.5% 3 log10,15.5% 4 log10, 25% 5 log10, 14.30% 6 log 10, and 4.6% 8 log 10 respectively. According to viral load, the depletion of CD4 was absolutely observed with percentages of CD4/CD8 and CD3. The conclusion of the study is that the 4th generation assay most accuracy than current 3rd generation assay in HIV acute stage screening and it should introduce in feature. The CD4/CD8 absolutes counts reference ranges must be established in each racial society and present studies established the reference ranges, it might useful for concerned population to access immunity in treatment. The HIV RNA viral load through Abbott assay is a major role in complete clearance of viral copy in antiviral treatment than others assay, especially HIV-1 genotypes inclusivity.

Key words: HIV RT-PCR, CD4/CD8 counts, CLIA.

Abbreviation: RT-PCR-Real Time Polymerase Chain Reaction, CLIA-Chemiluminence Immune Assay.

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1. INTRODUCTION

HIV/AIDS:
HIV is human immune deficiency virus infecting through sexual intercourse and blood transfusion. AIDS is acquired immune deficiency syndrome caused by HIV.

In the World, most of peoples have had died by immune deficiency virus (HIV) is a portion of the *genus lentivirus*, division of *Retroviridae* family contains 9200 nucleotide bases in its genome (International committee, 2006). It has originated from the Pan troglodytes *troglodytes*' species of chimpanzees (Gao et al.1999). It was accredited as an etiological origin of AIDS in 1983 (Montagnier et al.2002). The South-East Asia (SEA) is the next mostly bothersome WHO spot in the world, after sub-Saharan Africa. By the end of 2012, approximately 34 million people presently alive with HIV and nearly 30 million communities have died of AIDS after outbreak. Of these, 6.4 million are SEA region like Thailand, India, Indonesia and Myanmar. There are 14.8 million people eligible for HIV treatment; 8 million people were under the management of antiviral therapy and 7 million people not yet reached treatment in developed and developing nation (UNAID, 2012). The HIV occurrence radio is higher in those state like Manipur (1.40%), Andhra Pradesh (0.90%), Mizoram (0.81%), Nagaland (0.78%), Karnataka (0.63%) and Maharashtra (0.55%) are among the state of India (nacoonline.org). In the year of 2010 end, there are more than three hundred thousand cases undergone for antiviral treatment in about 25 centers of Indian states (Fiona et al. 2011).

Up to date there are four groups of HIV-1 isolate have been well-known and grouped according to its ribonucleic acid sequence of M, N, O, and P types. Approximately, 90% of infection caused by HIV-1 group M all over world and it's further subdivided into nine subtypes (A-I) depended on the complete genome, which are biologically divergent (Dale et al. 1996). Group O is restricted to West-Central Africa and group N is extremely uncommon and is originated only in Cameroon (Plantier et al. 2009). The one more succeeding type of HIV-2 was isolated from AIDS patients in West African Country in the years 1986 and it was less pathogenic than HIV-1, derived from sooty mangabey the Cercocebus *atys atys* (Santiago et al. 2005). HIV can be transmittable through mother to fetus or blood to blood contract or sexual contract. The introduction of virus in human blood after six weeks, infected persons normally expands a short, severe diseases characterized by flu-like symptoms and related with high levels of viremia in the peripheral blood (Clark et al.1991).

Comparison:

The current laboratory algorithm is more advanced than old CDC recommended algorithm. New immune assay has improved 4th generation assay and more accurate than 3rd generation assay. The 4th generation assay has got ability to detect antigen and antibody of HIV in same time and 3rd generation could be detect only antigen. The FDA approved methods are more hence; the Abbott assay has ability to detect a specific genotype which is more important to know of pathogenic and antiviral treatment management.

Content:

All the information which I mentioned in this research papers were written or spoken about in a book, an article, a programme, a speech, etc,

RT-PCR:
Real time polymerase chain reaction is molecular technology for genes amplification including HIV gag gene.

CD4/CD8:
Cluster differentiates (CD) 4 and 8 are important T cell receptor molecules. Produces from all nucleated cells.CD4 is responsible for Major Histo Compatibility Class-ll and CD8 is responsible for MHC class-l. This T cells receptor molecule are responsible for first against foreign proteins including HIVp24 antigen.

HIV can be diagnosed by different clinical laboratory technology for screening, confirmatory and antiviral regime. The screening assay is intended to discover all infected persons with great level of technical sensitivity but this grade might generate some false negative results. The second most confirmatory assay like RT-PCR, western bolt (WB) and new immune assay (IA) are designed to detect HIV infected persons and confirm negative population with great accuracy and with few false positive results. Detection of circulating HIV foreign protein through antigen based assays like ELISA or EIA, RIA or IA, IFA and Western Blot; CD4&CD8 cells population by flow-cytometry and RNA and proviral DNA by NAT test. Antigens which are used in HIV diagnostic tests must be accurately, and generally are purified novel antigens from viral lysates, or antigens created through recombinant or synthetic peptide technology. The use of such antigens allows HIV screening tests to own both sensitivity and specificity. FDA certification must be approved for screening blood, diagnosis, or monitoring disease. Due to scientific advances, there are different types of generation assay have evolved from different times for diagnosis of a specific diseases. The first generation immunoassay is designed for viral lysate antigen detection; the second generation immunoassay is depending on synthetic peptides and recombinant antigens and detects only IgG class antibodies; the third generation immunoassay is designed for detection of IgM and IgG class antibodies; and new fourth generation immunoassay that detect both classes of antibodies (IgG/IgM) and HIVp24 core antigen in window phase. HIV cannot be differentiate through CDC laboratory diagnostic algorithm in the stage of acute infections and mistakenly acknowledged about 60% of HIV-2 infection as HIV-1, based on HIV-1 Western blot outcome. Hence, the new diagnostic algorithm achieved better than the WB and replaced at detection of HIV antibody positive persons, distinguishing acute HIV -1 infections, and diagnosing unsuspicious HIV-2 infections (CDC, 2011; Styer et al. 2011; Nasurallah et al. 2013).

There are different clinical laboratory marker are at hand to monitor HIV diseases progression and overall management of health of infected persons. Anemia is common hemoglobin disorder in AIDS as well as other infectious diseases like Mycobacterium complex can affect red blood cells and its O_2carrying capacity. HIV infected persons are generally concerned with neutrophil, WBC, CD4 and CD8 cell counts. Most of the antiviral, antibacterial, and anticancer drugs might cause bone marrow suppression and it might be leading to deficiency of complete blood counts components. Long time bleeding disorder is refer to thrombocytopenia is caused by several factors but in HIV infected population due to usage of antiviral drug the platelet count might be reduced than normal ranges (Firnhaber et al. 2010). The normal CD4 cell percentage in a strong immunity person is about 30 to 60, although a normal CD8 cell percentage is 15 to 40 per cent (Taylor et al. 1989). The normal radio of CD4/CD8 cell is about 0.9 to 3.0 or higher, there are at least 1-3 CD4 cells for every CD8 cell. Hence, HIV infected population this ratio may be too low and CD8 cells might be greater than CD4 cells (Giorgi et al. 1993). The deficiency of CD4 cells counts are related with different types of environment condition like viral and bacterial infection, parasite and MTCI (mycobacterium complex infection) sepsis, cancer, intravenous injection of foreign protein, undernourishment, over-exercising, pregnancy, normal daily variation, psychological stress, and social isolation then immunodeficiency of AIDS. Hence, CD4 cells count below 200cells/µl necessary to diagnosis of AIDS with known cases of HIV. For anti viral treatment, need an average CD4 cells count below350 cells/µl and it was currently recommended by HIV treatment guideline groups (Walensky et al. 2010).

As an option of CD4 and CD8 + T cells lymphocyte count for HIV diseases monitoring, there are another parameter like β2-microglobin, Neopterin, IL2 receptor, IgA, and interferon (IFN) α and γ levels. The β microglobin is an 11kD protein expressed on the surface of most nucleated cells and Neopterin derived from guanosine triphosphate through de novo biosynthesis and it is an early marker for HIV infection. Free β2 microglobin can be calculated in both serum and urine levels of urine β2-microglobin associate with progression of AIDS. Also, the levels of β2-microglobulin are elevated in a different condition like lymphocyte proliferative syndromes, autoimmune diseases, renal diseases and viral infection (CBS Publisher, 1992). In addition, the routine diagnostic parameters like hematology profile, blood biochemistry profiles, and other routine test can assist continue to follow-up of diseases managements. The diagnostic report must be from same referral lab up to ending of treatment if it is not followed from same laboratory there is difficult to predict and decision making on antiviral treatment because of an unfair report (Gerber et al. 2003). Recent publication was mentioning about HPV are associated with early infected HIV population and it might be cause genital and anal cancer. This disease progression higher in infected of HIV population and it can be diagnosed through pap smears as well as supplementary test like RT-PCR of digene HC2 DNA assay.

An advanced confirmatory molecular diagnostic assay is HIV RNA quantification refers to the amount of genetic material of viruses which are circulating in serum or plasma of human blood. The blood tests of viral load indicate the number of copies or international unit of genetic materials of HIV RNA per milliliter of blood. Quantification of viral load results can range from less than 10 to million or billion copies and units. Diseases progression might be observed through viral load and prognosis of viral diseases could be suppressed by antiviral therapy. The immune system might be weakened by over replication of viruses result in depletion CD4 counts. If there is more viral load detected in blood, there are also probably to be high levels of virus in other fluid like semen or vaginal fluid. Hence, people with higher viral loads are expected to be more prone of viral infection. People with untraceable virus might also persist to be infectious; because viral load in blood, semen and vaginal fluid does not correlate correctly. Even though the risk of mother to child transmission is very low in women with undetectable viral load, transmission can occur. Likewise, it is possible to pass HIV to a sexual partner through contact to semen or vaginal fluid, although having an undetectable viral load (Calmy et al. 2004; WHO, 2006).

Generally, HIV viral load stable at a least ranges in women after sero-conversion. Among men, a least viral load after infection is associated with less risk of diseases progression. Most of current research declaration shows that woman's viral loads are notably lesser contrast to men with the same CD4 count. This might explain on the basic of mode of transmission and superior activity of hormones when compared to men. Most clinical practice guideline might follow the patterns of high viral load with low count of CD4, high CD4 count with low viral load or both markers could be higher with diseases progression. This might be depends on age groups, immunity and multiple opportunistic infection. Within six months of seroconversion, the CD4 count generally decreases about 30% and the CD8 count may increase by about 40%, resulting in an inverted ratio that is generally less than 1. With antiretroviral treatment (ART), the ratio may revert toward 'normal'. Long-term non-progresses and those who start antiretroviral treatment early on generally have a normal CD4/CD8 ratio. Also, further advanced diagnosis like genotypic, sequencing and

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phenotypic assays are for multi drugs resistant monitor, to be able to supervise AIDS scenario. In general, HIV RNA quantification and others routine diagnosis must be followed every 3 to 6 months intermediate if the infected persons are in ART (Koenig et al. 2006). Present study was focused on viral load of HIVRNA to assessment of antiviral treatment for suppression of viral replication in feature, quantification CD4/CD8+ T cells for predicting AIDS patient's immune condition and detection of HIV p24 antigen for screening of blood in the locality.

2. SCOPE OF STUDY

Acquired immunodeficiency syndrome is distinguished by CD4 reduction, CD8+ T cell increase and severe immune activation. To diagnosis and management of this virus infection, there are different methods are present in clinical laboratory like screening of antigen and antibodies by CLIA, absolute counts of CD4+CD8T cells lymphocyte by FACS and HIV RNA viral load by Abbott RT-PCR for antiviral monitoring therapy and approved by FDA for current medical practice. This study is suggested that the 4th generation assay most accuracy than3rd generation assay for HIV antigen and antibody detection in HIV-1&2 acute stage screening and it should introduce in feature. The CD4/CD8 counts reference ranges must be established in each ethnic society and present studies established reference ranges it might useful for concerned population to access immunity in treatment. The HIV RNA quantification through Abbott assay is a major role in complete clearance of viral copy in antiviral treatment than others assay especially HIV-1 genotypes inclusivity.

CLIA: Chemiluminence immune assay is technology for detection of antigens and antibodies.

FACS: Fluorescence activated cell sorting system for CD series counts.

2.1. Materials

2.1.1. Patients

This prospective scientific work was integrated 76 females and 273 males' suspected patients for HIV. The data has been received with background history of virus infection, from different city of Chennai as well as Tamil Nadu; patients referred by hospitals, doctors, and others laboratory centers. The samples were received by Hi-tech lab in T.Nagar branch for routine and supplementary diagnosis, during the year of 2010 to 2012. The ethics panel and internal review board of the organization approved the procedure. Informed authority was obtained from individual patients.

2.1.2. Specimen Collection

Approximately 10ml blood samples were received from each patient for different diagnosis. EDTA blood samples were used for hematology and cell counting analysis. Rest of blood sample were used for the separation serum, and plasma according to the test immediately by using centrifugation at 3000 rpm for 10 minutes and then each specimens was then aliquots into screw capped vials by labeling specific test name. These specimens were utilized for detection and quantification of HIV RNA and HIV p24 antigen. Statistical analysis was performed using Microsoft office excel 2007.

2.2. Methods

2.2.1. HIVp24 antigen quantification

Serum or plasma sample (100µl) of patients were applied to detect HIV p24 antigens by Cobas Integra 400plus clinical biochemistry analyzer (CLIA) with third generation kits, which is approved by FDA and center for infectious diseases (CID), and supplied by Roche Germany. The test results were releases within 40 minutes.

2.2.2. CD4/CD8 cells quantification

EDTA blood samples(1ml) were used directly after collection, for formative the absolute counts of CD4+, CD8+, CD3+T cells and their percentage by fluorescence activated cell sorting (FACS) count system (Becton Dickinson Pvt. Ltd., Mountain View, CA), using fluorochrome labeled monoclonal antibodies to CD4+/CD3+ and CD8+/CD3+ T-cells, seriously following manufacturer's directions. FACS count protocol software recent versions (Becton Dickinson) were used for data acquisition and analysis. The instrument was calibrated daily by using controls before the beginning of the work for sensitivity and specificity. Also, two different samples of one healthy and one HIV-positive case were included in each procedure and reproducibility was confirmed.

2.2.3. Real Time Polymerase Chain Reaction (Abbott)

Abbott Real Time HIV-1 clinical diagnostic assay is an in vitro reverse transcription-polymerase chain reaction technique for the quantification of HIV RNA in plasma sample by the automated m2000rt instrument, over the ranges of 10^1 to 10^8copies/ml. There are four major process involved for HIV RNA quantification such as RNA extraction, cDNA conversion by reverse transcriptase reaction, cDNA amplification and quantification.

2.2.4. HIV-1 RNA extraction

According to manufacture guideline, the RNA was extracted. The mAbbott2000™ Sample preparation system is a magnetic particle technology to capture nucleic acids and washes the particles to remove boundless sample components. The bound nucleic acids are eluted and transferred to a 96 deep well plate. Quality control sample have been included through the entire procedure.

2.2.5. HIV-1pol gene amplification

After HIV RNA extraction, 50ul of extracted RNA and PCR master mixer like oligonucleotide, thermo stable rTth polymerase enzymes and activation solution was added to a 96 well plate manually and loaded into system for amplification. During amplification reaction, the target RNA is converted to cDNA by the reverse transcriptase activity of the thermo stable DNA polymerase enzymes with help of cDNA PCR master mixer and extended through long time incubation. Further it is denatured the cDNA: RNA product to anneal a second set of target primer and extended by the DNA polymerase activity to get double stranded DNA product. Every PCR cycles, amplification products detach into single strands at higher temperature permitting primer annealing and extension as the temperature is lowered. Utmost amplification of the result is achieved through repeated cycling between high and low temperature, resulting in a billion-fold or better amplification of HIV target gene sequences. Amplification of both control and targets take place all together in the same reaction.

2.2.6. Detection

Two types of fluorescent dyes were used in this procedure. The reporter dye is concerned with gene amplification process and quencher dye is concerned with termination of end of amplification cycling for quantification. HIV-1 probe has a fluorescent moiety that is covalently linked to the 5 end. This quencher oligonucleotide is complementary to the 5 end of the HIV-1 probe and has a quencher molecule at its 3 end. The HIV-1 probe fluorescence is quenched through hybridization to the quencher oligonucleotide without target sequence and in same time with being there of the HIV-1 target sequence, the HIV-1 probe preferentially hybridizes to the target sequence, dissociating from the quencher oligonucleotide, allowing fluorescent detection. The HIV-1 and control specific probes are each labeled with a different fluorophore, thus allowing for real-time detection of both amplified products at each cycle.

2.2.7. Quantification

A calibration curve is needed to quantitative the HIV-1 RNA concentration of specimens and control. Two assay calibrators are run in replicates of three to generate a calibration curve in each kits being used. The calibration bow grade and cut off are calculated from the assigned HIV-1 RNA absorption and the median observed threshold cycle for each calibrator is calculated from the stored calibration

Table 1
Comparative data analysis of viral load quantification, CD4/CD8 depletion counts with p24 antigen detection

Viral load Copies/ml	Lymphocyte Counts		CD4 Counts		CD8 Counts		CD3 Counts		P24
	Abs	%	Abs	%	Abs	%	Abs	%	
TND-50ns (14.30%)	940-2590	25-37	510-1440	38-75	225-1020	20-53	900-2340	65-76	Ne-
TND-46ns (13.30%)	3200-7100	38-63	830-3400	36-75	430-1700	22-64	1990-5200	57-72	Po+
<40 -27ns (7.7%)	780-4100	15-50	91-1100	16-47	250-1750	46-80	380-2300	53-70	Po+
≤1000 -19ns (5.5%)	350-3100	15-34	80-2800	24-37	140-950	16-60	230-2350	62-80	Po+
≤10000-54ns (15.5%)	400-3255	11-35	11-890	4-35	223-1344	54-90	250-2357	46-80	Po+
≤100000-87ns (25%)	470-4000	16-55	59-850	9-45	250-2400	40-90	350-3000	49-86	Po+
≤1000000-50ns (14.30%)	450-2500	15-46	10-350	4-28	290-1400	66-92	315-1850	63-84	Po+
10000000-16ns (4.6%)	450-750	9-26	10-140	3-21	250-550	66-85	315-720	65-80	Po+

Table 2
Depletion of CD4 counts during acute infection between male and female

Age/ Sex	Viral load Copies/ml	Lymphocyte Counts		CD4 Counts		CD8 Counts		CD3 Counts		WBC
		Abs	%	Abs	%	Abs	%	Abs	%	
30F	23,52,838	720	11	33	6	418	76	546	76	6380
34F	13,71,694	740	12	48	9	320	68	520	74	4965
44F	10,88,372	700	15	123	24	339	66	514	73	4670
35M	20,44,966	880	32	56	9	543	85	638	73	2790
40M	44,90,204	1090	26	86	12	503	70	719	66	4120
42M	51,31,806	900	17	65	10	538	83	648	72	5210

curve, and the results are automatically reported on the system monitor.

3. RESULTS

A total of 349 suspected cases were included in this mega study with 50 negative sera as controls. Off these, 273 (78.23%) were males population and 76 (21.77%) females suspected cases. All of these cases were been screened through primary and secondary confirmatory assays and before it were screened through by clinicians from different part of Chennai Cities as well as Tamil Nadu. High risk age groups (25-40 years) have been observed in male and female suspected population and it were higher percentages in male (71.26%) when correlative with female infected cases through sexual exposure (21.4%). Rest of age groups between males and females (19-24years & 51-70 years) has been concluded that very low risk HIV infected persons but even though the age groups of 41-55 years old guys might be considered as second high risk HIV infected population. There are fifty negative cases were confirmed by both RT-PCR and 3rd generation immune assays out of 349 suspected cases. Besides that, 13.30 % (46/349ns) were target not detected by RT-PCR, while it was positive as p24 antigen by 3rd generation CLIA. The others three cases were confirmed as positive by RT-PCR, were it was negative by CLIA 3rd generation assay. Rests of the suspected cases were confirmed as HIV infected persons through RT-PCR as well as primary screening assay. The CD4/CD8 counts were accurately observed for each patient according to viral load and it was absolutely coordinated the CD4 depletion when the viral load and CD8 counts higher. The second high risk age groups between 35 to 50 years were highly viral replicate (>log10^8) then others age groups in this study. The actual viral load quantification were obtained as results in different ranges like 14.30% target not detected, 7.7%(< 40 copies/ml), 5.5% 3 log10,15.5% 4 log10, 25% 5 log10, 14.30% 6 log 10, and 4.6% 8 log 10.According to viral replication, the depletion of CD4 were absolutely observed with absorbance and percentages of CD4/CD8 and CD3. There was no correlation regarding age groups in this concern, especially CD4/CD8 counts (Table 1).The absolute depletion of CD4 counts during replication of virus were been observed in all HIV infected populations (Table 2). The 3rd generation chemiluminence immune assay has been designed for detection of HIV-1 IgG and IgM antibodies. The new fourth generation immune assay has been designed for detection of HIV-1&2 IgG, IgM antibodies and p24 antigen in same time. Current HIV diagnostic algorithm of CDC cant differentiate of HIV-1&2 but the new diagnostic algorithms might be more accuracy and can differentiate both HIV-1&2 (Figure 1).

4. DISCUSSION

Currently there are many methods available in molecular diagnostic market to control HIV diseases progression.FDA and US approved methods like Roche Cobas Amplicor HIV-1 Monitor Test version 1.5, based on target hybridization; Roche TaqMan HIV-1 Monitor Test depend on target specific dual labeled oligonucleotide probes with RT-PCR, Abbott HIV-1 Monitor Test rely on nucleic acid based target amplification; BioMerieuex NucliSens HIV-1 QT assay depend on isothermal nucleic acid amplification; Aptima qualitative assay (Gen-Probe) depend on target amplification and Bayer versant HIV-1 RNA version 3.0 assay based on branched DNA signal amplification. Whereas first generation assay quantitative viral load up to range of 400 copies/ml, the ultrasensitive latest generation tests in well-known clinical use today have a minimum detection range near 50copies/ml; a few assay can measure as few as 5 copies/ml. The maximum quantitative viral load obtainable through older generation assay, while the present generation assay great accuracy in low viral load quantification(Abbott/Taqman-10IU/ml).HIV-1 genotypes assortment is an important point to be considered for the improvement of genomic amplification method, usually for HIV plasma RNA quantification. HIV-1 non –B subtypes (CFR02-AG) strains are infected in newly diagnosed patients in Europe (France) and US and it is major role in antiviral treatment (Taylor et al. 2008). To achieve this process, most of the assays like Cobas TaqMan/Abbott, NucliSens updated assay were able to amplify equally of HIV-1 genotypes including non-B subtypes (Planella et al.1998). In same time most of assays were not capable to identify and measure HIV group O isolate and HIV-2 but merely Abbott/TaqMan assay has a great specificity to detect and quantification of viral load of HIV-1 group M subtypes A-H, Group N and O not HIV-2 have been observed.

Flow cytometry is an established method for quantification of total count of CD3+, CD4+ and CD8+ T lymphocytes. Absolute quantification of T -lymphocytes total counts are supported above percentages by both medical societies and Laboratories scientist (MMWR Recomm Rep, 1992; Forrest et al.1988). The perfect understanding of the results of this diagnosis depends on the sensitivity and specificity of method applied and the presence of test reference ranges of the counts in healthy population. In natural immune response, CD4 T cells react to antigen which is presented by Major Histo Compatibility Class II protein and Class I protein associated with CD8 +T cells (Doyle et al. 1987; Norment et al.1988). The CD4/CD8 ratio is between 1 and 3 an indication of immune system strength. Generally, normal population might have more CD4+ T cells population than CD8+ T cells and it might changes in its

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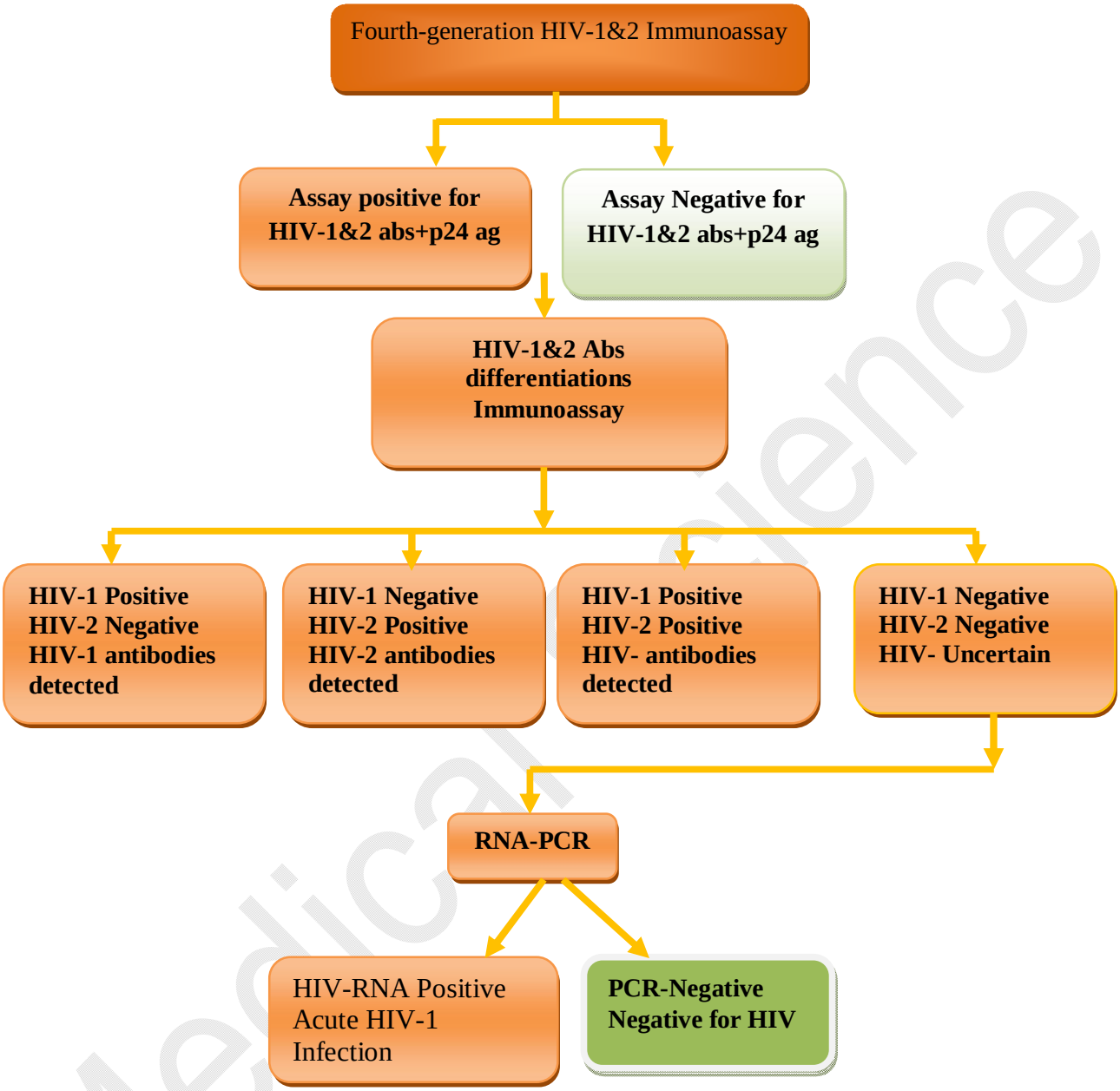

Figure 1

New HIV testing algorithm (2013)

ratio depends on age groups and geriatric persons. These counts might be higher in case of auto immune diseases and lower in viral infection but in anti viral therapy, these counts could be increased. The CD4/CD8 radios of less than 0.15 were more likely to have an immune reconstitution inflammatory syndrome (IRIS) than 0.30 radios (Ratnam et al. 2006). More counts of CD4+Tcells is determined by a permutation of homeostatic response to CD4 T cell depletion and viral load. In same time, CD8 +T cells propagation is obsessed mainly by HIV RNA viral quantification (Catalfamo et al. 2008).

The normal CD4 counts/µl of healthy Indian population has been published in different part of India as 848±395 cells/µl in North Eastern region-Manipur (Singh et al. 2000); 865 cells/µl in Western region-Pune; 1048±210cells/µl in Southern region-Vellore (Kannangai et al. 2000); 703±225 cells/µl in North region-Delhi (Krishna Ray et al. 2006) and present study from Chennai (Southern India-T. Nadu) analyzed as normal ranges1440±510 cells/µl. The current CD4 absolute quantification was totally different from others study which was published in different Indian states. The present study might be compared with International country like in Britons 830±290 cells/µl; Israelis 864±235 cells/µl and in Thais 910±310cells/µl, these have been published by scientist (Bofill et al.1992; Kalinkovich et al.1998; Vithayasai et al.1997). All of these standards were lesser to that of current ideal analysis. The preceding published data showed the reference ranges from 250-1510x10^6/l (Uppal et al.

2003) and this published data were correlated with present study. The absolute count of CD4 during beginning stage of HIV infection in female might be suddenly decreases when compare with male infected population and in same time once it is coming to average levels of viral replication it is balanced the CD4 counts because of hormonal variation than in male population.

The normal CD8 absolute counts of healthy population of Chennai were 1020±225 cells/µl in present study. Comparatively, very low levels in others region of India as well as which was reported earlier in Vellore (Kannangai et al, 2000). Absolute total counts of CD4+T cells was similar between normal and HIV infected patients and HIV RNA viral load< 40 copies/ml, considering very low viral load in these groups. In distinguish, the absolute total counts of CD8+T cells was higher in the HIV patients and HIV RNA viral load <40 copies/ml compared with negative population, signifying the incidence of an unending viral replication or at least continual force energetic CD8 +T cells activation in these studies with undetectable viremia. In early infection of HIV, the absolute quantification of CD4+ T cells are quiet wiped out from circulation when diseases progress and in same time CD8+T cells absolute quantification be likely to increase, impressive spreading out of memory CD8+Tcells in HIV infected persons (Roederer et al. 1995 & Margo lick et al. 1995). The current study was absolutely correlated with this earlier reported. The Architect HIV-1&2 Ag/Ab Combo method (Abbott Diagnostic) is new fourth generation immune assay (IA) posses higher sensitive in early detection of acute infection and differential diagnosis of HIV-2 infection than old generation assays like ELISA, EIA, RIA, IFA and WB (Styer et al.2011; Nasrullah et al.2013). The FDA approved HIV-1/HIV-2 Multisport (Bio-RAD) assay also similar rapid method for screening blood.

EIA: Enzymes Immunoassay, RIA; Radioimmunoassay, IFA: Indirect-immuno-fluorescence assay, IA: Immunoassay, WB: western blot.

5. CONCLUSION

The present study recommending the fourth generation immune assays are up dated version to detection of early acute phase of HIV infection. Flowcytometric analysis of single platform assay is best for CD4/CD8 counts in HIV/AIDS management and also established reference ranges. Abbott/TaqMan assays are top most technology than other assay, especially in detection of HIV-1 diversity including non B subtypes. The current study also highlighted the prevalence of HIV infection in Cosmopolitan city of Chennai, Tamil Nadu, India.

SUMMARY OF RESEARCH

1. CLIA is clinical diagnostic assay (3rd generation) for primary screening of HIV in all over the world. However, the new Immune Assay has more sensitive and specificity when compare with old assay so need to update according to current treatment practice for maintain human life.
2. There are different types of technical platform for immune calculation like CD4/CD8 for AIDS/HIV affected population. For this, the current research might helpful with reference standard for specific population and this single platform FACS more sensitive than other.
3. The viral load quantification is more important for decision making in antiviral treatment, especially the Abbott assay, which is more sensitive than another methods in HIV detection and quantification. The methodology might be useful for all affected population, generally who are in antiretroviral treatment.

FUTURE ISSUES

Adequate screening is important to control HIV infection. Saving of HIV infected population by antiretroviral treatment is good for Nation as well as economic growth of Universal.

DISCLOSURE STATEMENT

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