ORIGINAL RESEARCH

To Study HIV Seropositivity In Association With Various Dermatological Conditions

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ABSTRACT

Background: The most common cause of HIV disease throughout the world is HIV-I and HIV-2 which was first identified in 1986 in West African patients in which HIV-I subtype C is most prevalent. First case in India was identified in 1986 at Chennai. **Objective:** To study the correlation of different dermatological conditions with HIV infection and to Significance of ELISA for detection of AIDS (HIV infection) in patient of skin disease. **Methods**: The study was carried out on the patients of skin diseases attending Skin & V.D. outdoor clinic of L.L.R. & Associated Hospitals, Kanpur. The serum separated from the sample was then tested for antibodies to HIV at zonal blood testing centre, Blood Bank & Department of Pathology, Medical College, Kanpur. **Results:** Maximum percentage of cases belonged to 21-50 years of age group (77.7%). 5% of cases were recipients of blood transfusion while majority of cases 95% cases had no history of blood transfusion. 11.04% cases accepted for sexual promiscuity while 88.96% denied. This result was obtained despite of repeated enquiries. The clinical diagnosis of cases were candidiasis (28.13%), dermatophytosis (14.17%), genital scabies (12.08%), herpes zoster (11.25%), genital warts (7.08%), molluscumcontagiosum (7.91%) psoriasis (6.25%), syphilis (5.62%), seborrhoeic dermatitis (3.33%) , lichen planus (2.91%) and pyoderma (01.25%). Three cases of widespread candidiasis infection were found to be HIV seropositive. They were poorly responding to treatment. **Conclusions:** Prevalence of HIV seropositivity is about 1.25% of various skin diseases. However, a larger study with greater number of cases and longer duration is required to find out more concrete results.

Keywords: Prevalence, HIV, Seropositivity, Dermatological Conditions

INTRODUCTION

AIDS is a debilitating and life threatening disease and till date there are no drugs to cure it or a vaccine to prevent it. HIV is transmitted by both homosexual and heterosexual contact, by blood and blood products and by infected mothers to infants either intrapartum, perinatally or via breast milk. There are 2 major targets of HIV: the immune system and the central nervous system. HIV causes profound immunosuppression primarily affecting cell mediated immunity. Dermatologic problems occur in more than 90% of patients with HIV infection. The cutaneous manifestation of HIV may vary from macular to roseola like rash seen with the acute seroconversion syndrome to extensive end stage Kaposi's sarcoma.

An HIV rash often occurs with people that have contracted the HIV virus. An HIV skin rash is also caused by medications that are used to control the HIV virus. It is estimated that up to 90 percent of people infected with the HIV virus will develop some type of skin problems or skin condition during the length of the infection.

Skin rash can be caused by various sources and the sign of an unusual skin rash does not always mean an HIV infection. If you are in a high risk group and are experiencing some of the symptoms of HIV infection then a diagnostic HIV test will be able to confirm whether or not you have contracted the virus.

Early HIV symptoms of an HIV infection develop with a couple of weeks to two months after contracting the virus. The symptoms mimic the flu: fever, headaches, swollen gland, muscle aches and pains, and loss of appetite. In addition, an I-11V rash develops on skin. An infected individual may not necessarily experience these early symptoms. As the HIV infection progresses the symptoms change and this can take up to nine years for some people. The later symptoms include: extreme fatigue, regular unexplained fevers, weight loss, constantly enlarged lymph nodes, frequent diarrhea, painful and stiff

joints, memory loss, vision problem and constantly contracting bacterial, fungal and a viral infections.

In terms of the skin, the HIV symptoms include a HIV rash on the skin which is often flaky in nature like a severe case of psoriasis, sores around the male or female genital areas, easy bruising of the skin, warts that are rather large in size on feet, hands, face, anal and genital regions and mouth sores or lesions inside the mouth. Hives in large patches that are very itchy can also develop. Folliculitis and acne like pimples may form around hair follicles on most skin areas of the body. An HIV rash can may also occur because of medication that is used to control or treat HIV infection.

Hence this study was conducted to Correlation of different dermatological conditions with HIV infection and to Significance of ELISA for detection of AIDS (HIV infection) in patient of skin disease.

MATERIALS AND METHODS

The study was carried out on the patients of skin diseases attending Skin & V.D. outdoor clinic of L.L.R. & Associated Hospitals, Kanpur. Criteria of selection of cases was based on clinical diagnosis of various skin diseases. With the consent of the patient the blood sample was collected. The serum separated from the sample was then tested for antibodies to HIV at zonal blood testing centre, Blood Bank & Department of Pathology, Medical College, Kanpur.

COLLECTION OF SAMPLE

1. The blood was drawn by disposable syringe and needle and collected in sterilized plain vial All due precautions were taken to avoid contamination at any stage.
2. Only human serum or plasma samples should be used for the test. While preparing serum samples, remove the serum form the clot as soon as possible to avoid hemolysis. Fresh serum /plasma samples are preferred.
3. Specimens should be free of microbial contamination and may be stored at 2-8°C for one week, or frozen at -20°C or lower. Avoid repeated freezing and thawing.
4. Use of heat inactivated, icteric, hyperlipemic and hemolyzed samples should be avoided as may give erroneous results. Transportation
5. If the specimen is to be transported, it should be packed to compliance with the current Government regulations regarding transport of aetiologic agents.

MATERIAL REQUIRED

a) Patient sample
b) HIV kit
c) Distilled or deionised water
d) Presion pipette
e) Disposable tips
f) Multichannel pipette
g) Microplate washer
h) Absorbent tissue
i) Microplate reader
j) Incubator

PRINCIPLE OF THE TEST

When patient serum contain HIV antibody combines with HIV peptides attached to polystyrene surface of micro strip wells. Residual patient sample is removed by washing and horse radish peroxidase conjugated antihuman immunoglobulin is added, well are washed and a colorless enzyme substrate hydrogen peroxide and chromogen (tetra methyl benzidine) are added, the enzyme reaction of substrate—hromogen produces a color end product enzyme substrate chromogen reaction is terminated with sulfuric acid. The color intensity is directly related to the concentration of HIV antibodies in the patient serum and is measured by ELISA reader at specific wavelength.

PREPARATION OF REAGENTS

Prepare the following reagents before or during assay procedures. Reagents and samples should be at room temperature (20 — 30 °C) before beginning the assay and can remain at room temperature during testing Return reagents to 2-8°C after use. All containers used for preparation of reagents must be cleaned thoroughly and rinsed with distilled or deionized water. Pre warm the incubator at 37°C.

I. HIV Strip:

Bring foil pack to room temperature (20-30°C) before opening to prevent condensation on the micro well strips.

a) Break off the required number of strips needed for the assay and place in the well holder. Take the strip holder with the required number of strips, taking into account that two negative and three positive controls should be included in the run while opening the fresh kit. However, for one or two strips, one negative and two positive controls and for more strips at least two negative and three positive controls should be included in each subsequent runs.
b) Unused wells should be stored at 2 — 8°C with desiccant in a aluminum pouch with clamp and rod.

Caution: Handle micro well strip with care. Do not touch the bottom exterior surface of the wells.

2. Sample Preparation:

1. **Tube Dilution:** Mark the tubes carefully for the proper identification of the samples. Dilute the serum samples to be tested with sample diluents (1 : 11 dilution in separate tubes (200µl diluents + 20 µl sample) . Use a separate tip for each sample and then discard as biohazardous waste.
2. **Microwell Dilution:**

a) Pipette 100111 of sample diluents in to the mirowell
b) Add 10111 of serum sample to be tested
c) Ensure thorough mixing of the sample to be tested.

3. Preparation of wash buffer

a) Check the buffer concentrate for the presence of salt crystals. If crystals are present in the solution, resolubilize by warming at 37°C until all crystals dissolve.
b) Prepare at least 50 ml (2 ml concentrated buffer with 48 ml water) of buffer for each microlisa strip used. Mix well before use.
c) Mix 20 ml 25 X wash buffer concentrate with 480 ml of distilled or deionized water. Wash buffer is stable for 2 months when stored at 2-8°C.

4. Preparation of Working Conjugate:

Dilute conjugate concentrate 1:100in conjugate diluents. Do not store working conjugate. Prepare a fresh dilution for each assay in a clean glass vessel. Determine the quantity of working conjugate solution to be prepared.mix solution thoroughly before use .

Preparation of working substrate solution — mix TMB substrate and TMB diluents in 1:1 ratio to prepare working substrate.

WASH PROCEDURE

1. Aspirate the well content completely into a waste container , then fill the wells completely with wash buffer avoiding overflow of buffer from one well to another and allow to soak (approx. 30 seconds). Aspirate completely and repeat the wash and soak procedure 4 additional times for a total of 5 washes.
2. Automated washer if used should be adjusted to fill each well completely without overfilling.
3. Incomplete washing will adversely affect the test outcome.
4. Tap upside down on absorbent sheet till no droplets appear on the sheet. Taking care not to dislodge the wells.

CALCULATION OF RESULTS

Absorbance of negative control: 042(Blwell) + 0.040 (Clwell)
0.082 (2well)
Mean negative control: 0.041
Absorbance of positive control:
1.412(Dlwell) + 1.392(Elwell) + 1.407 (Flwell)
Mean positive control: 1.403
Cut off value 0.041+1.403/6 = 0.240

RESULTS

The study of HIV seropositivity in cases of various skin diseases was conducted on 480 cases. These patients were attending to the out door clinic of skin ad VD at L.L.R. and Associated Hospitals, Kanpur.

77.7% of cases belonged to the age group of 21 – 50 years while 11.2% of cases belonged to age group of more than 50 years and 10.9% of cases belonged to age group of less than 20 years.

According to marital status of patients 69.79% patients were married while 30.21% were unmarried

Out of 480 patients 86.4% belonged to Kanpur proper while 13.6% of patients were from places around kanpur like Unnao and Kanpur Dehat.

Table 1: Distribution of cases according to blood transfusion

Blood transfusion history (B.T.)	Cases	Percentage
History of B.T. present	24	05
History of BT absent	456	95
Total	480	100

Out of 480 patients 95% of patients did not receive blood transfusion while 05% of patients had history of blood transfusion.

Out of 480 patients, only 2 patient had history of IV drug abuse.

Table 2 – Distribution of cases according to intravenous drug abuse

I.V. Drug abuse history	Number of cases	Percentage
History of IV drug abuse	02	0.42
No history of IV drug abuse	478	99.58
Total	480	100

Table 3: Distribution of cases according to Multiple sexual contacts

History of multiple sexual contacts	Number of cases	Percentage
H/o multiple sexual contacts present	53	11.04
H/o multiple sexual contacts not present	427	88.96
Total	480	100

Majority 88.96% of patients did not give the history of multiple sexual contacts while only 11.04% cases gave the history of multiple contacts.

Table 4: Distribution of cases according to clinical diagnosis

Diseases	Number of cases	Percentage of cases
Candidiasis	135	28.13

Dermatophytosis	68	14.17
Genital Scabies	58	12.08
Herpes zoster	54	11.25
Genital warts	38	7.91
Molluscumcontagiosum	34	7.08
Psoriasis	30	6.25
Syphilis	27	5.62
Seborrheic dermatitis	16	3.33
Lichen planus	14	2.91
Pyoderma	06	1.25

Common clinical diagnosis wereCandidiasis, dermatophytosis, genital scabies, herpes zoster, genital warts.

Table 5: Distribution of cases according to clinical diagnosis subjected for hiv testing (Detection of anti HIV antibodies by ELISA)

Clinical diagnosis	Number of cases positive for anti HIV antibodies / Total number of cases	Percentage
Candidiasis	3	0.62%
Dermatophytosis	-	-
Genital Scabies	-	-
Herpes zoster	2	0.42%
Genital warts	-	-
Molluscumcontagiosum	-	-
Psoriasis	-	-
Syphilis	-	-
Seborrheic dermatitis	-	-
Lichen planus	-	-
Pyoderma	1	0.21%
Total	6	1.25%

Out of 480 patients 6 patients (1.25%) were positive for anti HIV antibodies. 4 positive patients were male and2 were female they found positive on repeating the ELISA test.

DISCUSSION

In our present study, age group of 10-60 years is taken into consideration in which maximum number patients that is 77.7% fall under the age group of 21-50 years. At the same time, **Jing**[1] done a retrospective study in Malaysia found majority of patient in age group of 20-50years. **Uthaya et al**[2] studied the patients under the age group of 25-50years.

Above mentioned studies show approximately the same age group and are matching with our study. However, many studies are retrospective and few are prospective but pattern of age taken into consideration were same. Mean age varies according the age group taken by the above respective studies.

In present study out of 480 cases 76.5% are males and 23.5% cases are females, **Van de perreet at**[3] **(1988)** included 131 patients in their study,among them 64.9 % were male and 35.11% were female. Above mentioned studies matched with the present study two shows male preponderance. Among them two studies are different , under which one have 100% male while other one have 100% female in their study. Although it is wholly depend on the choice of observer but in our study, general population attending the skin OPD's are taken in consideration and we found that ratio of male: female.

Out of 480 cases 69.79% cases were married and 30.21% cases were unmarried. This is the usual pattern of marital status in the general population in the age groups included in our study.

70% cases belonged to Kanpur city while only30% were from places around Kanpur. That were belonging to nearby district e.g. Unnao, Kanpur Dehat etc. This pattern is representative of geographical area which has easy access to L.L.R. Hospital Kanpur.

The present study showed only 95% cases had received blood transfusion while **Healy et al**[4] **(1993)** found 8.6% cases had received blood transfusion the value are more or less similar to the present study.

In the present study only 0.42% are intravenous drug abuser. In **Garbeet et al**[5] **(1994)** study 12.7% patients were drug abusers while in **Healy et at**[4] **(1993)** 67.39% were positive for drug abuse. 7.4% i.v. drug abusers had been. studied by the **Munoz et al**[6] **(1998)**. This major discrepancy might be due to higher prevalence of drug abuse in the western countries.

In the present study 11.04% patients gave the history of sexual contact. **Munoz et al**[6] **(1998)** evaluated a group of patients, among them 14% were given the positive history while in study of **Garbe et al**[5] **(1994)** 77.9% gave the positive history. In study of Garbe c et at history of multiple sex partner is very high, the reason behind this was that they studied HIV positive

patients with various cutaneous diseases while our study is dealing with patients of various dermatological conditions who might show seropositivity to HIV.

The common diseases for which patients consulted the skin clinics in the present study were candidiasis (28.13%) dermatophytosis, (14.17%), genital scabies (12.08%), herpes zoster (11.25%), genital warts (7.08%), molluscumcontagiosum (7.91%) psoriasis (6.25%), syphilis (5.62%), seborrheic dermatitis (3.33%) , lichen planus (2.91%) and pyoderma (01.25%). These patients were not responding to treatment and subjected to testing for HIV. **Munoz et al**[6] **(1998)** retrospectively studied 1161 cases with HIV infection. They found skin disease in 799 patients with HIV infection. They found oral candidiasis, seborrheic dermatitis, herpes zoster were the most common infections.

Singh et al[7] **(1998)** studied 125 pateints out of which 50 were positive with HIV infection. In their study mucocutaneous diseases in order of frequency were: candidiasis, dermatophytoses, herpes simplex, oral aphthaexerosis, scabies, HPV infection, molluscumcontagiosum. **Jing & Ismail**[1] **(1999)** found that 130 out of 180 patients with HIV infection had mucocutaneous disorders (71.4%). They found most common diseases were oral candidiasis (35.7%), seborrheic dermatitis (19.2%), tineacorporis and onychomycosis (9.9%) psoriasis (7.7%) and herpes infection (4.3%). **Healy E**[4] **(1993)** et al seen a wide spectrum of skin disorders in patients with HIV infection. They found a significantly higher prevalence of seborrheic dermatitis, Kaposi's sarcoma, oral candidiasis, folliculitis, molluscumcontagiosum, onychomycosis, herpes simplex in the HIV positive groups compared to control groups. **Menendez and Milian**[8] **(1992)** seen forty Cuban HIV positive patients with various cutaneous diseases. They found herpes simplex, oral candidiasis multidermatomal herpes zoster, dermatitis seborrhoeica and onychomycosis were common diseases. **Hira et al**[9] **(1988)** found 1124 HIV infected patients with one or more cutaneous diseases. In their study Kaposi's sarcoma multidermatomal herpes zoster, pruritic maculopapular rashes, candidiasis, severe genital herpes, extensive molluscumcontagiosum and tineacorporis were frequent dermatologic manifestations. **Sindrup et al**[10] **(1988)** encountered most frequent skin diseases in HIV positive patients were oral hairy leukoplakia (2.1%) dermatophytosis (including tineaunguim / tineapedis et inguinalis) (20%) seborrheic dermatitis (19%) viral infections (10%), oral candidiasis, acne vulgaris (6%), folliculitis (5%), herpes zoster (3%).

Most of the authors studied the skin diseases in HIV. However as our study is concern with seroprevalence of HIV in skin diseases. **We found 6 cases out of 480 patients were seropositive for HIV infection.**

In present study we found 3 out of 135 patients of oral and genital candidiasis were positive for HIV infection. Association of candidial infection in HIV disease appears to be very significant in our study as well as in the existing literature.

Next two patients who shown HIV positivity, suffering from multidermatomal herpes zoster. Last, sixth patient with HIV seropositivity was suffering from pyoderma.

This study matched with the observations made by. **Munoz—Perez et al**[6]**(1998), Jing and Ismail** [1]**(1999), Singh et al** [7]**(1998), Menendez and Milian**[8] **(1992), Hira et al** [9]**(1988),** they all found that more or less oral candidiasis was the most frequent skin disease present in HIV positive patients. Second number is that of herpes zoster and third is the pyoderma.

The variability of results are seen because most of the workers have selected HIV positive cases, they classify the presence of skin disease after wards while the present study criteria of selection of cases in skin diseases of various groups and they are subjected for seropositivity of HIV. However, larger study for a longer period is required in this prospective for more concrete results.

CONCLUSION

It is concluded that prevalence of HIV seropositivity is about 1.25% of various skin diseases. However, a larger study with greater number of cases and longer duration is required to find out more concrete results

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