Estimation of salivary secretory leukocyte protease inhibitor level in HIV infection

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Abstract:
Salivary secretory leukocyte protease inhibitor (SLPI) is an antimicrobial protein found in saliva. The concentration of SLPI has shown to increase in HIV positive patients suggesting its anti HIV activity. The SLPI level in saliva was estimated in HIV positive patients and compared with healthy subjects. Unstimulated (whole) saliva was collected from 30 confirmed HIV positive patients and 30 healthy subjects .and levels were estimated in both groups. The salivary leukocyte protease inhibitor levels were significantly raised in HIV positive patients as compared to healthy subjects with a P value of 0.019. Salivary secretory leukocyte protease inhibitor was significantly increased in HIV infection. .
Keywords: Salivary secretory leukocyte protease inhibitor, HIV.

Introduction:
In today's era of modern technology, medical research has emerged with remedies to conquer most of the health problems yet a few pose challenges to the mankind, human immunodeficiency virus (HIV) infection being one of them. HIV infection is the cause of concern world wide as it has resisted most of the treatment efforts ever since its advent. India has the world's second largest burden of HIV

infection. It has devastating health, social, psychological and financial consequences.[1]

Initially it was proposed that HIV might be transmitted casually from the oral secretions of HIV carriers during kissing, dental treatment, biting and aerosolization. Further research proved that oral transmission of HIV infection was rare, in spite of virions being isolated from saliva and gingival crevicular fluid. The antiviral activity of saliva was attributed to the presence of various factors in saliva which inhibit HIV-I infection. Among these proteins, human salivary secretory leukocyte protease inhibitor (SLPI) was one which was capable of inhibiting HIV-I replication in vitro at physiologic concentration .Human secretory leukocyte protease inhibitor is an 11.7-kda cationic protein and a member of the innate immunity-associated proteins. It is a nonglycosylated, highly basic, acid-stable, cysteine-rich, 107-amino acid, single-chain polypeptide.[2.]

Saliva is currently proving to be an effective, economical and easily available tool for diagnosis. There are very few studies comparing the level of salivary SLPI among HIV infected patients and healthy subjects.

It was also observed that addition of the protease inhibitors to the antiretroviral regime delayed the progression of disease and also to certain extent decreased the oral complications. Limited literature is available regarding level of SLPI in HIV patients.

The present study was therefore undertaken to assess the SLPI level in HIV positive patients and then compared with healthy subjects.

Materials and Methods:

Source of Data: The present study was carried out in the Department of Oral Medicine and Radiology, KLES's Institute of Dental Sciences, Belgaum. The study group included 30 HIV positive patients and 30 healthy age and sex matched patients visiting the OPD of KLES's Institute of Dental Sciences, Belgaum and Spandan Organisation (Non-Government Organization), Belgaum. Each of the participants in this study granted informed consent.

The patients were divided into following groups

Group A: 30 HIV positive patients.

Group B: 30 age and sex matched healthy individuals.

Exclusion Criteria:

1. Subjects with bacterial and fungal infections (Control group B)

Methodology:

The entire methodology has been divided into 5 stages.

1. A detailed case history was documented
2. General physical and oral examination was carried out.
3. Collection of samples: An informed consent was obtained from patients willing to participate in the study. Saliva samples were collected by aseptic procedure in all the groups. 5ml of unstimulated whole saliva was collected by spitting method into a 50ml plastic screw capped container.
4. Pretreatment and storage of samples: The samples were stored at -70°C immediately after collection until their transportation to laboratory for sample pretreatment.
5. SLPI levels in saliva of HIV patients and healthy subjects were detected by using SLPI ELISA kit (Hycult Biotechnology). The test was carried out in microbiological laboratory.

Biological Principle of the Test:

The human SLPI ELISA test kit is a solid phase enzyme linked immunosorbent assay based on the sandwitch principle. Samples and standards are incubated in microtiter wells coated with antibodies recognizing human SLPI.

During this incubation, human SLPI was captured by solid bound antibody. Unbound material present in the sample was removed by washing. Next biotinylated second antibody tracer to human SLPI was added to the wells. In the presence of SLPI in sample, the tracer antibodies bind to the captured SLPI. The excess tracer was removed by washing. Next a streptividin-peroxidase conjugate was applied to the walls, this conjugate results specifically with the biotinylated tracer antibody bound onto the detected SLPI. The excess

streptividin-peroxidase conjugate was removed by washing and substrate tetramethylbenzidone (TMB) was added to the wells. Color develops proportional to the amount of human SLPI present in the sample. The enzyme reaction was stopped by the addition of citric acid and the absorptions at 450mm was measured with spectrophotometer.

Evaluation of Results:

1. The average absorbance values (A_{450}) for each set of duplicate standards, samples and controls was calculated and analysed.
2. If sample absorbance values differed by more than 15% from the corresponding mean value, the sample was re-assayed.
3. The mean absorbance value of the zero standard in controls was less than 0.3.
4. A standard curve by plotting the mean absorbance for each standard on the vertical (Y) axis versus the corresponding SLPI concentration on the horizontal (X) axis was constructed.
5. Using the mean absorbance value for each sample, the corresponding concentration of SLPI from the standard curve was determined. The absorbance value was plotted on the Y-axis and an horizontal line was extended to the curve. From the point of intersection a vertical line was extended to the X-axis and the SLPI concentration was read for the unknown sample.
6. In diluted samples the concentration determined from the standard curve was multiplied by the dilution factor.

Stastical Analysis:

Mean Standard Deviation and Mann Whitney U Test formula was used.

In all above tests "p" value of less than 0.05 was accepted as indicating statistical significance.

P value was considered in the following manner
Not significant > 0.05
Significant < 0.05
Very Significant < 0.01
Highly significant < 0.001

Calculation diluted by 100ml
1pkgm = 100ng   Concentration x ng / 1000 = µg (1ng=1000µg)

Results and Observations:

In group A maximum number of patients were in the age range of 26-35 years (66.7%) followed by 15-25yrs (26.7%) as shown in (graph no.1) .

The groupA comprised of 16 male patients i.e. (53.3%) and 14 females i.e. (46.7%) as shown in (graph no.2)

The group B (control group) consisted maximum number of patients in age range of 15-25 years (75.87 %) followed by 26-35(20.6%) and 1 patient which was in 36-45 age range (graph no 3).The Group B included 23 male and 7 females. (Graph no 4)

The SLPI concentration in the group A was in the range of 0.1-0.2(3.33%), 0.3- 0.4 (30%), 0.5 to 0.6µg/ml in 18 patients (60%) and 0.7 - 0.8 (6.67%) as shown in (graph no5.)

Among the healthy subjects (group B) SLPI level was in the range of 0.0- 0.1 (25%), 0.1-0.2 (75%) , 0.3-0.4(none) as shown in the(graph no 6.)

The comparative analysis of SLPI level in both the groups was statistically significant with a P value=0.0019 obtained with Mann Whitney U Test. This clearly indicates that there was a significant rise in the level of SLPI among study groups as compared to control group as shown in(table no 1.)

**Table No.1: Comparison of SLPI level in HIV positive patients
and control group**

| Sl No	HIV +ve Patients			Control Group		
	Age	Sex	SLPI Conc.	Age	Sex	SLPI Conc.
1	26	F	0.524	21	M	0.126
2	35	M	0.645	17	M	0.164
3	35	M	0.597	24	M	0.132
4	22	F	0.475	23	M	0.129
5	24	F	0.489	23	M	0.110
6	25	F	0.480	22	M	0.102
7	32	M	0.491	21	M	0.670
8	28	F	0.605	18	M	0.940
9	26	F	0.565	23	M	0.109
10	25	F	0.564	22	M	0.196
11	25	F	0.538	22	M	0.880
12	35	M	0.437	22	M	0.123
13	26	F	0.553	19	M	0.900
14	33	M	0.281	22	M	0.890
15	24	M	0.602	30	M	0.880
16	28	F	0.741	22	M	0.117
17	33	M	0.704	22	M	0.104
18	35	M	0.657	32	M	0.114
19	32	F	0.531	21	M	0.610
20	40	M	0.580	20	M	0.610
21	32	M	0.482	42	M	0.104
22	30	M	0.479	24	F	0.132
23	35	M	0.485	25	F	0.123
24	35	F	0.475	24	F	0.133
25	26	F	0.550	26	F	0.107
26	28	M	0.506	26	F	0.114
27	25	F	0.567	28	F	0.133
28	38	M	0.551	22	F	0.114
29	15	M	0.546	28	M	0.128
30	30	M	0.503	30	M	0.283
MEAN 0.5401					MEAN	0.3101

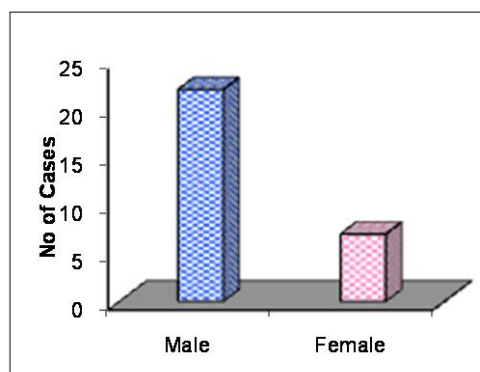**GRAPHICAL PRESENTATION:**
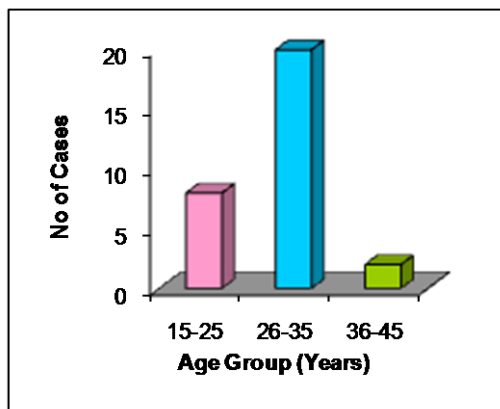
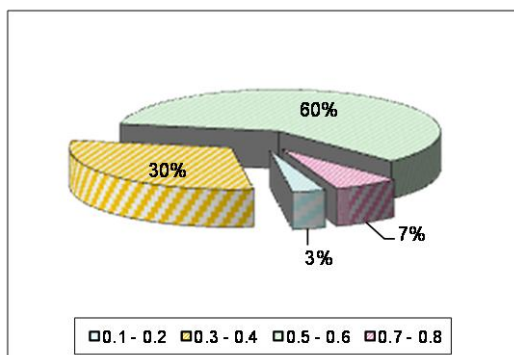**Graph No.1: Age distribution of HIV**
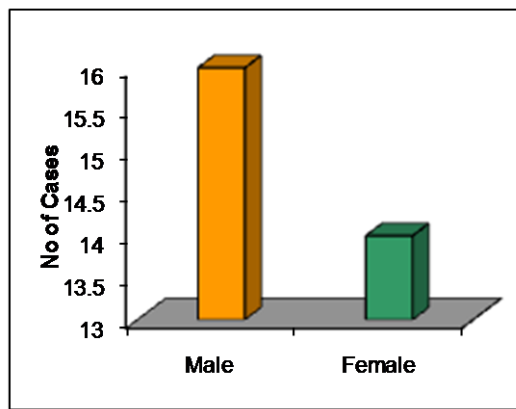
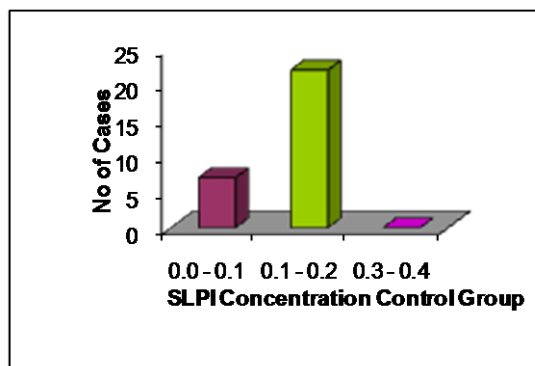**Graph No.2: Sex distribution of HIV positive.**
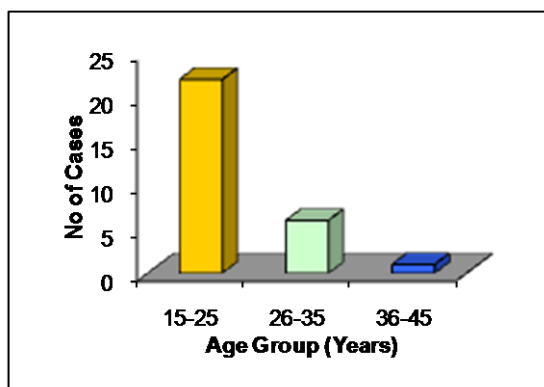
Graph No.3: Age distribution in control

Graph No.4: Sex distribution in control

Graph No.5: Graph showing percentage of SLPI level in HIV positive patients

Graph No.6: SLPI level in

Discussion:
HIV infection has emerged as one of the leading challenges to global health ever since its advent in

1981. The modes of transmission has become more and more complex, hence it is imperative to know the common routes of transmission for prevention

strategies as the incidence of reported cases has increased drastically.

Oral transmission of HIV is rare as compared to other modes of transmission. This could be attributed to the presence of antiviral factors in saliva like Secretory Leukocyte Protease Inhibitor which may contribute in protecting Oro-pharyngeal mucosa against invasion by HIV-1. The exact role of SLPI in prevention of oral transmission is elusive, though studies have reported a rise in the SLPI level in patients with HIV infection.[3]

The present study was therefore carried out to assess the levels of SLPI in patients with HIV infection and was compared with healthy subjects.

SLPI has been found to be the most potent anti HIV factor in saliva among several innate inhibitory molecules, namely virus specific antibodies, mucins and thrombospondins. Human secretary leukocyte protease inhibitor is 11.7 kDa cation protein and a member of innate immunity associated protein secreted by acinar cells. The SLPI concentration was determined in 30 HIV positive patients and 30 healthy subjects from saliva sample using human SLPI ELISA kit. .

In the present study maximum patients were in the age group of 26-35 years (66.7%) followed by 15-25 years (23.3%). This is in accordance to a study carried by Yo et al[Ai(3)] wherein patients ranged between 25-60 years. Similar findings were also reported by AM Bquit where patients (1999)[4] were in the range of 29-59 years.

The numbers of male patients in our study were 16 (53.3%) and females were 14 (46.7%) with M: F ratio of 1.14:1. The increase in the number of male patients was consistent with other studies (Sudhir 1994)[5.] This could be attributed to increased practice of multiple sex partners among males. Also a lack of knowledge about spread and use of unsafe sexual practice in the rural areas could play a vital role as most of the male patients in the present study belonged to rural area.

The SLPI level in 18 patients among the study group was in the range of 0.5-0.6 mg/ml. This was consistent with the observation of Alan (2004)[6] who reported that SLPI levels were in the range of 0.5 to 0.6μg. The SLPI levels in 23 healthy subjects in our study ranged from 0.1 to 0.2μg/l. Thus the SLPI levels were significantly raised in HIV positive patients as compared to healthy subjects. The P value obtained was 0.0019, thus indicating a statistically significant difference in the SLPI levels among the two groups. Similar findings were also reported by Alan Dorthy[6], Chal He Lee,[7] Dijkornan JH et al [8]where in SLPI level were increased by 75% in HIV positive patients as compared to healthy subjects.

The exact role of SLPI in reducing the risk of transmission is still elusive. A few authors have hypothesized that increased salivary concentration could be the result of decreased fluid secretion which could be attributed to factors like xerostomia, medications, gland inflammation and smoking habits. Bquit et al also proposed that antiviral activity of SLPI could be due to inhibition of a protease mediated event that is required for virus entry and infectivity of susceptible cells.[,9, 10]

The present study shows a higher level of SLPI in HIV positive patients. This increase in SLPI level in saliva account for higher anti HIV activity, by reducing the risk of oral transmission of HIV.

It is essential to know that higher SLPI levels protect against HIV infection supports further research so as to understand the exact mechanism of action of SLPI in saliva in reducing the risk of oral transmission. This could facilitate to develop new prevention strategies of HIV infection and also strengthen our understanding of mucosal immunity.

Conclusion:

The present study clearly indicates a definite rise in Salivary Leukocyte Protease Inhibitor level in HIV positive patients as compared to healthy subjects. .

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