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# Salivary pH and buffering capacity in early and late human immunodeficiency virus infection

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## ABSTRACT

**Background:** Human immunodeficiency virus (HIV) causes severe immunosuppression due to progressive decrease in the CD4 T lymphocyte cells during the course of the disease and this affects all the body systems including glandular secretions. A number of lesions affecting the salivary glands have been noted in HIV infection. The objective of this study was to evaluate the salivary pH and the buffering capacity in HIV positive individuals and comparing it with the HIV negative healthy individuals.

**Materials and Methods:** The study was carried out on 200 HIV positive subjects aged 20-40 years, divided into two groups on the basis of CD4 count and 100 HIV negative healthy individuals as control group. Both unstimulated and stimulated saliva were collected and the pH and buffering capacity ascertained using the saliva check kit. (GC Asia Dental Pvt. Ltd., Singapore, 508724).

**Results:** All the three groups were compared using the ANOVA and it was found there was highly significant decrease in pH and buffering capacity with increase in immunosuppression. The intergroup comparison was carried out using the Tukey honestly significant difference (HSD) and the Chi square test. Group 1; CD4 count <200 and Group 2, CD4 count >200 showed a significant decrease in unstimulated salivary flow, stimulated salivary flow, and pH in comparison to HIV negative individuals; however, change in buffering capacity in Group 2 was not significant.

**Conclusion:** There is a decrease in pH and buffering capacity in HIV infected patients. This decrease may be one of the factors responsible for increased caries in HIV infected population.

**Key Words:** Buffering capacity, human immunodeficiency virus, pH, salivary gland disease

## INTRODUCTION

A number of lesions affecting the salivary glands have been noted in human immunodeficiency virus (HIV) infection. These lesions are characterized by enlargement of the major salivary glands, symptoms of dry mouth or both.[1]

Patients with HIV-salivary gland disease (SGD) and parotid gland swelling have significantly reduced stimulated parotid flow rates compared with HIV-seronegative control subjects.[2] HIV cannot be cultured from the saliva of patients with HIV-SGD despite positive culture from the blood of the same patients.

A preliminary study of the sialochemistry revealed increased albumin and borderline increase of IgA, protein, and lysozyme in HIV-SGD.[3]

The human mouth is quite frequently exposed to components whose pH differs from the saliva's normal pH. These components may cause damage to the teeth or mucosal surfaces. Buffering function try to bring the pH back to the normal range as fast as possible.[4-6]

The present study aimed to investigate changes, if any, in pH, and buffering capacity in different stages of HIV infection.

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## MATERIALS AND METHODS

The study was carried out over a period of 19 months from November 2010 to June 2012. A total of 200 HIV positive patients, divided into two groups on the basis of CDC classification for HIV infection.[7]

- AIDS patients with CD4 count <200/cu.mm
- HIV infected patients with CD4 count >200/cu.mm
- 100 healthy individuals yielding negative HIV ELISA test were also included.

Ethical clearance was obtained from the Institution Review Board. Patients were informed about the objective of the study and signed an informed consent form before participating in the study.

All patients were refrained from eating, drinking, smoking, and performing oral hygiene procedures for 2 h before saliva collection. Standard precautions for health-care workers laid down by World Health Organization were followed. All samples were collected over 5 min, between 9:00 a.m. and 11:00 a.m.[8] The saliva sample analysis was carried out using the saliva-check kit(GC Asia Dental Pvt. Ltd., Singapore, 508724).

### Collection of unstimulated saliva

Subjects were comfortably seated and after a few minutes of relaxation, they were trained to avoid swallowing saliva and asked to lean forward and spit all the saliva they produced for 10 min in a graduated cup.

### pH measurement

The patient was instructed to expectorate any pooled saliva into the collection cup. A pH test strip was taken and placed into the sample of resting saliva for 10 s and then the color of the strip was checked. This was compared with the testing chart available in the package.

### Collection of stimulated saliva

For collecting the stimulated salivary sample, the subjects were asked to fast for a period of 1 h, sample was taken in a relaxing and sitting position, they were made to chew a basic gum tablet, which comes with the kit, with the objective of stimulating salivation. All the saliva accumulated in the first 30s was discarded (swallowed or expelled).

### Buffering capacity

A Buffer test strip from the foil package is removed and placed onto an absorbent tissue with the test side up. Using a pipette, sufficient saliva from the collection cup was drawn, and dispense one drop onto each of the 3 test pads. The test pads began to change color immediately and after 2 min the final result was calculated by adding the points according to the final color of each pad.

- Conversion table,
- Test pad color at 2 min,
- Green 4 points,
- Green/blue 3 points,
- Blue 2 points,
- Red/blue 1 point,
- Red 0 points,

The result was interpreted.

Combined total Buffering ability of saliva

- 0-5 very low,
- 6-9 low,
- 10-12 normal/high.

## RESULTS

The average age of both HIV infected and AIDS disease group was 33 and HIV negative healthy individuals was 31 years old [Table 1].

There were 58% males and 42% females in AIDS infected group, 52% males and 48% females in HIV infected group, 55% males and 45% females in HIV negative healthy individuals [Table 1].

In the AIDS infected group, 40 out of 100 patients and in HIV infected group, 52 out of 100 were on anti-retroviral therapy, but it was not found to be significant [Table 2].

The SPSS 18 software program was used for statistical analysis.

The mean pH for AIDS infected patients, HIV infected population, and the HIV negative healthy individuals were found to be 5.952, 6.036, and 6.802 respectively. The ANOVA test was carried out and

**Table 1: Age and gender distribution in AIDS infected, human immunodeficiency virus positive and human immunodeficiency virus negative group**

| | Aids infected (*n*=100) | Hiv positive (*n*=100) | Hiv negative (*n*=100) |
|---|---|---|---|
| Age distribution | | | |
| 20-30 Years | 47 | 42 | 48 |
| 30-40 Years | 53 | 58 | 52 |
| Gender | | | |
| Males | 58 | 52 | 55 |
| Females | 42 | 48 | 45 |

the *P* value showed very high significance [Table 3, Figure 1].

The inter group comparison was carried out using the Tukey HSD. The results between AIDS infected group and HIV negative healthy individuals, HIV infected group and HIV negative group were found to be very highly significant, but in between AIDS diseased population and HIV infected persons was not significant [Table 4].

82% of the AIDS patients had low buffering capacity, HIV infected patients had 58% with normal buffering capacity and HIV negative group had 70% with normal buffering capacity [Figure 2]. The ANOVA test was carried out and *P* value <0.001 was recorded, which showed very high significance [Table 5, Figure 3].

The inter group comparison using the Tukey HSD yielded very high significant results between AIDS patients and HIV infected individuals, AIDS infected and HIV negative population, but difference between HIV positive group and HIV negative group was not significant [Table 6].

## DISCUSSION

Although salivary volume and composition are constantly changing, repeated salivary samples collected under strictly standardized conditions seem to be fairly constant in the same individual. This is especially true for paraffin stimulated flow rate and buffering capacity values.[5,6]

It must be pointed out that the saliva research literature on HIV individuals does not refer much on

**Table 2: Effect of anti retroviral Therapy Art on variables studied using the chi-square test**

| Art therapy | | | | |
|---|---|---|---|---|
| Art therapy | Count % | Group | | Total |
| | | Aids infected | Hiv positive | |
| N | Count | 74 | 80 | 154 |
| | % | 74% | 80% | 38.5% |
| Y | Count | 26 | 20 | 46 |
| | % | 26% | 20% | 11.5% |
| Total | Count | 100 | 100 | 400 |

a.X2=0.508, *P*=0.476 ns

**Table 3: Comparison of unstimulated saliva pH between AIDS infected, human immunodeficiency virus positive and human immunodeficiency virus negative using the ANOVA test**

| | N | Mean | STD. Deviation | F | *P* |
|---|---|---|---|---|---|
| Ph | | | | | |
| Aids infected | 100 | 5.9520 | .67680 | | |
| Hiv positive | 100 | 6.0360 | .79764 | | |
| Hiv negative | 100 | 6.8020 | .78804 | 28.09 | <.001 vhs |

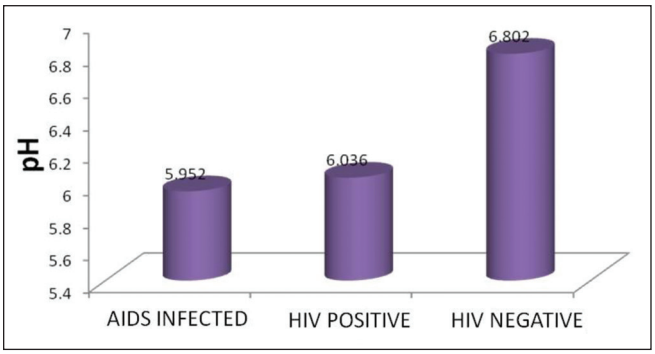


**Figure 1:** Depicting the mean unstimulated saliva pH values in AIDS infected, human immunodeficiency virus positive and human immunodeficiency virus negative group

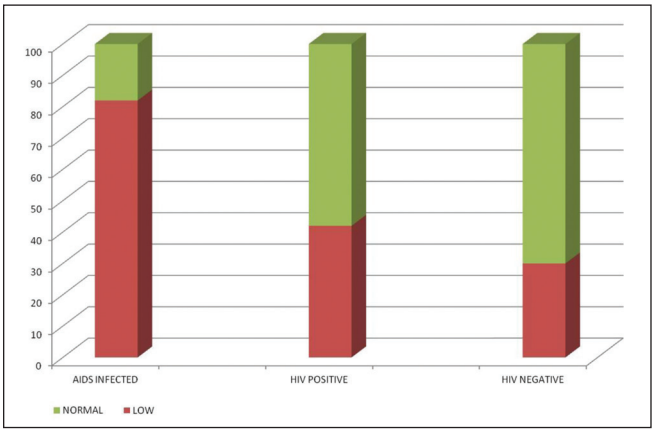


**Figure 2:** The percentage distribution of individuals with low stimulated saliva buffering capacity in AIDS infected, human immunodeficiency virus positive and human immunodeficiency virus negative group

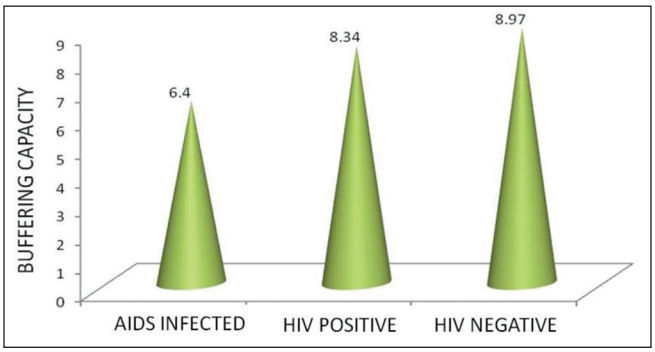


**Figure 3:** Mean stimulated saliva buffering capacity values in AIDS infected, human immunodeficiency virus positive and human immunodeficiency virus negative group

**Table 4: Intergroup comparison of unstimulated saliva pH using the Tukey HSD test**

| | | Tukey HSD | | |
|---|---|---|---|---|
| **Dependent variable** | **(I) Group** | **(J) group** | **Mean difference** | ***P* value** |
| pH | Aids infected | Hiv positive | –.0840 | .847 |
| | Aids infected | Hiv negative | –.8500 | <.001 Vhs |
| | Hiv positive | Hiv negative | –.7660 | <.001 Vhs |

**Table 5: Comparison of stimulated saliva buffering capacity between AIDS infected, human immunodeficiency virus positive and human immunodeficiency virus negative group using the ANOVA test**

| | | Tukey HSD | | |
|---|---|---|---|---|
| **Dependent variable** | **(I) Group** | **(J) Group** | **Mean Difference (I-J)** | ***P* value** |
| pH | Aids infected | Hiv positive | –1.9400 | <.001 Vhs |
| | Aids infected | Hiv negative | –2.5700 | <.001 Vhs |
| | Hiv positive | Hiv negative | –.6300 | .335 |

**Table 6: Intergroup comparison of stimulated saliva buffering capacity using the Tukey HSD test**

| | **N** | **Mean** | **STD. deviation** | **F** | ***P*** |
|---|---|---|---|---|---|
| Aids infected | 100 | 6.4000 | 2.74791 | | |
| Hiv positive | 100 | 8.3400 | 2.33527 | | |
| Hiv negative | 100 | 8.9700 | 2.58767 | 16.826 | <.001 Vhs |

salivary changes occurring in disease. There has been no research regarding buffer capacity and pH. For this reason, this study is fundamental to understanding the importance of saliva and its flow-dependent components in maintaining oral health.

In the present study, pH value was ascertained in the resting saliva sample using the pH strip. Watanabe *et al*. found that the method using the pH indicator strip is highly reproducible both within the samples and between examiner. They also confirmed agreement between the results obtained to pH strip and those obtained by pH meter by linear regression analysis. The overall accuracy of salivary pH measurements using the indicator strip was found to be high.[9]

In another study, it was found that there is a best agreement among results provided by strip-type systems in patients with high buffering capacity. However, certain disagreement of the buffering capacity was observed for patients with medium or low values.[10]

Correctly classifying a person's buffering capacity is critically dependent on the elapsed time after pipetting the saliva sample on to the strip. Carbon dioxide evaporates from the saliva sample and if longer than 5 min elapses after the pipetting, an increase in that individual's buffering capacity occurs. Although care was taken to read results within 5 min of sampling, nonetheless, a bias towards high readings might have been present.[10]

Both the HIV infected groups showed the mean pH nearly six, which is well above the critical pH of enamel. In contrast, the critical pH for dissolution of cementum and dentin is slightly above 6.0.[11,12]

In some studies, the critical pH for root surfaces was determined to be 6.2, which represents a six fold greater solubility for root surfaces compared with enamel.[13,14] Furthermore, there is gingival recession due to poor periodontal condition in HIV disease. This signifies that the HIV positive individuals are more prone for root caries and fast progression of dentinal caries once the lesion is in contact with oral cavity.

The AIDS infected group showed decrease in buffering capacity, which was significant when compared to HIV positive and HIV negative healthy population. Närhi *et al*., found that the Subjects with low salivary flow rates and low buffering capacities had significantly higher yeast counts than subjects with normal salivary flow rates and buffering capacities. This could possibly explain the higher incidence of fungal infection in HIV disease.[15,16]

HIV disease is characterized by immunosuppression, that is decrease in the CD4 count of the infected people, the decrease in pH and buffering capacity may be attributed to the decreased immune-competence of the HIV infected individual as has been found in other immuno-compromised conditions.[17]

It can be concluded that there is a decrease in pH and buffering capacity in HIV infected patients. Although AIDS patients; CD4 count <200 show a significant decrease in both pH and buffering capacity, HIV positive individuals; CD4 count >200 showed a significant decrease only in pH.

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