Oral health among HIV infected paediatric patients in Stockholm County.

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Summary

Aim
Oral manifestations are common among HIV infected children and adolescents. Conflicting reports have been published concerning the effect of antiretroviral (ARV) treatment on the oral status of HIV infected individuals. The aim of this study is to investigate the oral status of HIV infected paediatric patients attending the Department of Pedodontics at the Karolinska Institute in comparison to a control group of non-infected children attending the same clinic. Furthermore, the level of Candida among healthy children and adolescents in Stockholm was investigated.

Methods
A cross-sectional study based on a systematic review of the dental journals of 45 HIV infected patients attending the clinic during 2009-2011. Data concerning caries experience, oral lesions, stimulated salivary flow rate and microbial content were collected from the dental journals. Whole stimulated saliva samples were collected from a control group consisting of 71 healthy paediatric patients and analysed in regards to salivary flow rate and microbial content. Data concerning caries experience were collected from their dental journals. Obtained values were analysed using the SPSS 16.0, Chicago, IL.

Results
63% of the HIV infected group had positive Candida growth in the saliva. In the healthy control group the corresponding number was 34%. Salivary flow rate, caries experience and levels of Streptococcus Mutans and Lactobacillus in the HIV positive group were similar to the controls.
Conclusions

- 34% of the control group was asymptomatic carriers of *Candida*.
- The HIV positive patients attending the Department of Pedodontics at the Karolinska Institute during 2009-2011 had a good oral health concerning caries and oral lesions.
- Despite a good oral health and ARV treatment the HIV positive patients showed a significantly higher amount of salivary *Candida* (63%).
- The clinical significance and implications of elevated levels of *Candida* in HIV infected patients needs further investigation.
Authors’ contributions

Hanan Ahmad has been involved in;
- preparatory review of the literature
- planning the project and writing a project plan
- designing a database
- reviewing the entire dental journals of all HIV infected paediatric patients having attended The Department of Pedodontics at the Karolinska Institute since the 1990’s.
- analyzing and recording above data for all patients into the database
- organizing the collecting procedure of saliva samples from a control group and collecting saliva samples
- recording data from the control group into a database
- writing and submitting the paper.

Mina Tägt Khodabandehloo has been involved in;
- preparatory review of the literature
- planning the project and writing a project plan and a progress rapport
- designing a database
- reviewing the entire dental journals of all HIV infected paediatric patients having attended The Department of Pedodontics at the Karolinska Institute since the 1990’s.
- analyzing and recording above data for all patients into the database
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Introduction

According to the World Health Organization (WHO), in the end of 2010 an estimated 34 million people worldwide, whereof 3.4 million children <15 years of age, were living with HIV/AIDS. In the same year 1.8 million people died of AIDS related causes and out of these 250 000 were children. Due to the improved availability of medical therapy and consequently improved survival rate, people living with the virus have increased the last decade. At the same time the incidence of HIV infection continues to decrease. Sub-Saharan Africa remains to be the region worst affected, standing for approximately two thirds of the global HIV infection. (1)

HIV infection is yet incurable and the medications available today is used to halt the progression of the disease in order for the infected individual not to acquire AIDS. The medications, also referred to as antiretrovirals (ARVs) are aimed to improve the immune status of the infected individual and keep the viral load at an undetectable level. ARVs, at present, consist of five groups; nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PI), fusion or entry inhibitors and integrase inhibitors. A combination of three or more ARVs is referred to as highly active retroviral therapy (HAART). (2)

Oral manifestations may be one of the first signs of HIV infection in children (3). Since the oral cavity easily can be examined, it is of outer importance that the dentist possesses basic knowledge of the changes that could be expected from the infection.
There are many similarities between the oral manifestations seen in HIV infected children and those seen in HIV infected adults, although there are some differences in their incidence. Kaposi sarcoma, non-Hodgkins lymphoma and oral hairy leukoplakia are example of lesions strongly associated with HIV infection in adults (4,5) however rarely seen in children. Oral candidiasis, herpes simplex virus infection, linear gingival erythema, parotid enlargement and recurrent aphthous ulcers are manifestations often seen in paediatric patients. Lesions less commonly associated with the paediatric population are periodontal diseases, xerostomia, viral infections and bacterial infections of the oral mucosa. (5)

Oral candidiasis is reported to be the most common oral lesion in HIV infected children (3,6,7,8). The lesion is of great importance in its role as a marker for disease progression (8,9). It appears in three different forms; pseudomembranous, erythematous and angular cheilitis, the first-mentioned being the most common type (3,6,7,8). A prerequisite for oral candidiasis is colonisation of Candida spp. in the oral cavity. However, not all HIV positive subjects with positive growth of yeast show clinical signs of the lesion. Therefore, the presence of Candida spp. per se is not an indicator for oral candidiasis but should rather be seen as a risk factor when present in high rates. (10) Candida albicans is reported to be the most common Candida spp. found in HIV infected children (9,11). Pomarico et al. (12) similarly reported Candida albicans to be the most common type in the oral cavity of HIV positive children and their HIV negative siblings, although the species was found in the healthy group at a lower rate. Other Candida
spp. identified in their study were *C. parapsilosis*, *C. guilliermondi*, *C. Tropicalis*, *C. krusei*, *C. dubliniensis*, *C. glabrata* and *C. lusitaniae*.

There seems to be a positive correlation between ARV therapy and decline in oral symptoms of HIV infected children. Several studies suggest that subjects under medication have a reduced amount of oral candidiasis (7,12,13) and are not colonized with *Candida* spp. to the same extent as HIV infected children without medical treatment. Oral candidiasis appears more frequently in children with lower CD4+ T-lymphocyte counts compared to those with superior immune status (12). Provided that oral lesions are associated with low immune status (3,6) and high viral load their presence in subjects under medical treatment could therefore be a sign of treatment failure (6).

Children with HIV are reported to have greater dental caries experience in the primary dentition than non infected children (14,15,16). Meanwhile, it is proposed that higher caries prevalence in the primary dentition compared to age matched healthy children could be due to a delayed tooth eruption pattern in the permanent dentition in HIV infected children (17). A study carried out by Madigan et al. (15) demonstrates that HIV infected children >6 years of age are more prone to develop caries lesions than their uninfected siblings living in the same household. With no difference in carbohydrate intake, the HIV infected children in the study had more decayed tooth surfaces and fewer caries free surfaces. Greater experience of dental caries in some HIV positive children could furthermore be a result of a more advance stage of the disease (15,18). It is suggested that there is a
correlation between the presence of dentinal carious lesions and growth of *Candida* spp. in the saliva of HIV infected children, indicating that dentinal carious lesions per se could predispose yeast growth (11,19). A recent study (9) has in addition reported that children with higher levels of *Candida* colonisation present a higher amount of carious lesions. These findings indicate that absence of dentinal carious lesions could protect against yeast growth in the oral cavity of HIV infected children (9,11,19).

Parotid gland enlargement is one of the common oral lesions found in paediatric patients with HIV infection (3,5,7). In contrast to oral candidiasis it is associated with better prognosis (3,20) and a slower progression to AIDS (20). It occurs as a soft swelling and might be unilateral or bilateral. Xerostomia may occur simultaneously with parotid gland enlargement (5), although the association between the lesion and altered salivary flow rate remains controversial (13). Many of the reports are studies and review articles from the late 1980’s or early 1990’s (21,22,23) and carried out on adult study populations. Observations concerning the relationship between parotid gland enlargement and the salivary gland function in HIV infected paediatric patients appear rare. However there are studies regarding stimulated salivary flow rate carried out on healthy paediatric patients (24, 25,26), yet the obtained values point in different directions.

It is estimated that there are about 130 children and adolescents (0-18 years) infected with HIV living in Sweden today. Vertical transmission is the most common way of acquiring the virus (27). However, between the years of 2000 and 2010 only seven such cases are documented in Sweden (28). The
The majority of the HIV infected children and adolescents have been infected by the virus in their native countries before immigrating to Sweden. They all have the possibility of living relatively normal lives thanks to antiviral therapy, extensive apprehension by the health care, support groups and educational forums. (27)

The HIV infected children and adolescents in Stockholm County are registered at the Infection Clinic at Karolinska University Hospital Huddinge. Since the 1980’s many of them have been referred to the Department of Pedodontics at the Karolinska Institute for dental examinations and treatment. The patients have been examined every year with regard to dental caries, periodontal health, oral lesions, saliva function and level of *Candida* spp. in saliva. Despite good knowledge about the etiology, pathogenesis and treatment of the HIV infection, an overview of the oral status of these patients is not available today. Furthermore, data concerning salivary flow rate and salivary *Candida* in healthy Swedish children and adolescents seem to be rare in up to date studies. The aim of this study is therefore, to investigate the oral health status of HIV infected children and adolescents in Stockholm County. Additionally, it is of great interest to document parameters concerning salivary flow rate and levels of salivary *Candida* in a healthy control group. The hypothesis is that the HIV infected patients attending the clinic in general possess a poor oral health. They are expected to demonstrate mucosal lesions and higher rates of salivary *Candida* and carious lesions compared to a healthy control group.
Materials and Methods

This is a cross sectional study based on odontological and medical data documented in the patient notes of 45 HIV infected paediatric patients between the years of 2009 and 2011. Parameters investigated were compared to data collected from a control group consisting of 71 healthy paediatric patients between the years of 2010 and 2011.

Study group

The study group (HIV+) constituted all HIV infected patients that visited the Department of Pedodontics at the Karolinska Institute between the years of 2009 and 2011. In total there were 45 vertically infected HIV positive children and adolescents (24 male, 21 female), ranging from 7 to 20 years of age. All of them have been visiting the Infection Clinic at the Karolinska University Hospital Huddinge regularly and then been referred to the Department of Pedodontics at the Karolinska Institute for dental examinations. The patients have been examined and treated by specialists in paediatric dentistry.

The dental journals of the study group were systematically reviewed. Oral health variables from year 2009 to 2011 were collected and registered into a database in Microsoft Excel 2007.

Control group

The control group constituted 71 healthy children and adolescents (40 male, 31 female), ranging from 7 to 19 years of age. The patients in the control group have been receiving dental treatment at the Departments of
Pedodontics/Orthodontics at the Dental School of the Karolinska Institute and were selected with regards to their age to match the study group. Each age group constituted approximately five patients.

In the same manner as for the study group, oral health variables were collected from the patient notes and registered into a database in Microsoft Excel 2007.

Medical status was confirmed by anamnestic data, as told by the patient or guardian. Exclusion criteria were systemic diseases, any resent antibiotic treatment or administration of other drugs.

**Variables studied**

**Dental Caries**

Experience of caries was registered according to the DMF and dmf indices, the latter representing the primary dentition. D/d = decayed, defined as teeth with manifest (D3) and secondary carious lesions. M/m = missed, defined as teeth extracted due to caries lesion. F/f = filled, T/t = teeth, S/s = surfaces. Molars and premolars extracted due to caries were counted as 3 surfaces. Incisors and canines extracted due to caries were counted as 2 surfaces. Results were presented as DMFT/dmft, DMFS/dmfs, DT/dt and DS/ds.

The DMF and dmf indices were not available for some of the controls who only attended the clinic for orthodontic treatment.
**Oral lesions**

Mucosal manifestations as they have been described in the dental journals by the examiner. This information was not available for the control group.

**Saliva**

Saliva samples for the HIV+ group have been obtained by the treating dentist, and the values were collected from the dental journals. Whole stimulated saliva samples from the control group were collected by 4th and 5th year dental students at the Departments of Pedodontics/Orthodontics at the Dental School of the Karolinska Institute. Samples were taken at any time of the day. No food restrictions were given. The students were however instructed not to apply any Fluoride-varnish on the patients’ teeth before the saliva sampling.

The method used for collection of saliva samples was the same for the study group and the control group. All patients were asked to chew on a 1 g paraffin tablet for five minutes while expectorating all produced saliva into a plastic cup. Salivary flow rate was registered as ml/min. 2 ml of saliva was then accumulated using a measuring syringe (25ml) and added into a VMG II medium. Salivary flow rate was registered and the samples were thereafter sent to the Department of Laboratory Medicine at the Karolinska Institute and analysed with regards to *Streptococcus Mutans* (*S. Mutans*), *Lactobacillus (Lb)* and *Candida*. 
Microbiological analysis

Agitation of the collected specimen was carried out for 30 seconds by mixing in a Vortex. Thereafter, phosphate buffer was added to the sample to make dilutions of $10^{-1}$, $10^{-2}$, $10^{-3}$ and $10^{-4}$. 20 µL aliquotes of the dilutions were then applied on agar plates, each divided into four different sections. For cultivation of *S. Mutans*, Mitis Salivarius agar (Difco 298), with addition of sucrose and bacitracin, was chosen as growth medium. In the same manner, dilutions were dotted on a dried surface of Rogosa SL agar plate (Difco 480) for growth of *Lb*. The spots on the Rogosa plates were let dry after which additional SL agar was poured over the surface. The Mitis Salivarius agar was incubated at 37º for 48 hours in 95% N$_2$ and 5% CO$_2$. The Rogosa agar was incubated at 37º for 72 hours in an aerobic environment. (29)

Using the same dilutions as above, *Candida* growth was determined using a selective growth medium made of Sabouraud Dextrose agar (Difco 109). To avoid growth of bacteria and permit growth of fungi alone, a solution containing antibiotics (20 mg Gentamycin + 5 mg chloramphenicol) dissolved in 10 ml of 95% alcohol was added. The plate was incubated at 37°C for 24 hours aerobically.

The number of grown colonies was then counted. The lowest detectable values were 150 amounts of bacteria/ml saliva for *S. Mutans* and *Lb*, and 30 CFU for *Candida*. 
Children exhibiting more than $10^6$ amount of bacteria/ml for *S. Mutans* and $10^5$ amount of bacteria/ml for *Lb* were registered as having positive growth. The cut off point was made at these values, since they are reported to have a significant correlation to caries risk (30). Regarding *Candida*, positive growth was registered when the saliva sample presented yeast growth above the minimum detectable level of 30 CFU.

**Ethical Considerations**

This report is a Master’s dissertation and ethical considerations were taken into account and was approved by the department of Pedodontics at the Karolinska Institute. No procedures harmful to the patients were carried out. The patients in the control group and/or their guardians were informed about the study before giving verbal consent to their participation. All participants were coded to maintain anonymity and no identification of the participants was possible.

**Statistical Analysis**

A descriptive analysis of the obtained data was carried out with the statistical software program SSPS, version 16.0 (SSPS, Chicago, IL). Differences in the salivary and clinical parameters between the patient- and control group were analysed by $\chi^2$-squared test (categorized variables) and Mann-Whitney (numerical variables). Associations between variables were analysed by the Spearman rank order correlation analysis ($r_s$). The level of significance was accepted at $P<0.05$.  


Results

Forty five HIV infected individuals were included in the study group, out of which 24 were male and 21 female. All children were perinatally infected. Ages ranged from 7 to 20 years, with a mean age of 12.9 years (±3.4). Forty individuals underwent HAART, one medicated with only one PI and four individuals did not use antiretroviral medication at all. The medications had been taken for at least one year. The control group constituted of 71 healthy individuals, 40 males and 31 females. Mean age was 12.9 (±3.7) years, with ages ranging from 7 to 19 years. Variables studied are presented in Table 1.
Table 1. The variables studied among HIV+ and healthy children and adolescents.

<table>
<thead>
<tr>
<th>Variables</th>
<th>HIV+ Mean (s.d.) (n=45)a</th>
<th>Control Mean (s.d.) (n=71)a</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (F/M)</td>
<td>21/24</td>
<td>31/40</td>
<td>0.751a</td>
</tr>
<tr>
<td>Age (Years)</td>
<td>12.9 (±3.4)</td>
<td>12.9 (±3.7)</td>
<td>1.0c</td>
</tr>
<tr>
<td>DMFT/dmft</td>
<td>2.6 (±2.7)</td>
<td>2.5 (±3.0)</td>
<td>0.606c</td>
</tr>
<tr>
<td>DMFS/dmfs</td>
<td>3.7 (±4.4)</td>
<td>3.6 (±4.8)</td>
<td>0.683c</td>
</tr>
<tr>
<td>DT/dt</td>
<td>0.7 (±1.3)</td>
<td>0.6 (±1.6)</td>
<td>0.660c</td>
</tr>
<tr>
<td>DS/ds</td>
<td>0.9 (±1.8)</td>
<td>0.9 (±2.5)</td>
<td>0.584c</td>
</tr>
<tr>
<td>SSFR (ml/min)</td>
<td>1.3 (±0.7)</td>
<td>1.3 (±0.8)</td>
<td>0.691c</td>
</tr>
<tr>
<td>*Candida (Y/N)d</td>
<td>25/15</td>
<td>24/47</td>
<td>0.003b</td>
</tr>
<tr>
<td>*S.Mutans (Y/N)e</td>
<td>8/33</td>
<td>12/57</td>
<td>0.668b</td>
</tr>
<tr>
<td>*Lb (Y/N)f</td>
<td>3/38</td>
<td>11/60</td>
<td>0.182b</td>
</tr>
</tbody>
</table>

SSFS. Stimulated Salivary Flow Rate; Y, Yes; N, No
a Some data was not available for all patients, b χ2 as statistical method, c Mann-Whitney as statistical method.
d Growth ≥30 CFU. e Growth ≥10⁶/ml saliva. f Growth ≥10⁵/ml saliva.

Caries

DMF/dmf indices were available for 45 of the HIV infected patients and 61 of the controls. Figure 1 and 2 show the distribution of caries experience within the two groups. The average score did not differ significantly between the groups (P>0.05). Overall both groups had few manifest carious lesions with mean DS/ds scores of 0.9. The mean DMFS/dmfs scores were 3.7 (±4.4) for the HIV+ group and 3.6 (±4.8) for the control group.
Figure 1. Prevalence (%) of caries experience expressed as DMFS/dmfs in the HIV+ group (N=45) and the controls (N=61)

Figure 2. Prevalence (%) of caries experience expressed as DS/ds in the HIV+ group (N=45) and the controls (N=61)
Oral lesions

No oral lesions had been detected amongst the HIV infected patients.

Saliva

Values of stimulated salivary flow rate were obtained from 41 HIV infected patients and 66 controls. The mean stimulated salivary flow rate was 1.3 (±0.7) ml/min for the HIV+ group and 1.3 (±0.8) ml/min for the controls. No significant difference between the two groups was found (P>0.05).

Values for S. Mutans were available for 41 patients from the HIV+ group and for 69 patients from the control group. 19% of the HIV infected children and 17% of the controls had S. Mutans counts ≥10^6 bacteria/ml saliva. Lb counts were available for 41 HIV infected individuals and 71 controls. 7% within the HIV+ group and 15% of the controls had Lb counts ≥10^5/ml saliva. There was no significant difference in growth of S. Mutans and Lb in saliva between the two groups (P>0.05).

Candida counts in the stimulated saliva were available for 40 of the HIV infected patients and 71 controls. 63% of the children within the HIV+ group and 34% within the control group had a positive growth of Candida in saliva. The number of individuals with positive Candida growth as well as the average amount of Candida (mean CFU/ml saliva), was significantly higher in the HIV+ group (P=0.003). There was a large skew in the distribution of Candida counts within the HIV+ group. The minimum value was <30 CFU whereas the maximum value was 2.7x10^7 CFU. Such a big variation was not seen in the healthy control group. Figure 3 displays the
distribution of *Candida* over age in both groups. As shown, positive yeast growth increased with age within the control group.

**Figure 3.** Age distribution of detectable *Candida* spp. (>30 CFU/ml), in stimulated saliva among the HIV positive individuals (n=40) and controls (n=71).

A correlation analysis was carried out to determine a possible relationship between the total amount of carious lesions and *Candida* colonies. A positive correlation was found between amount of decayed surfaces (DS/ds) and *Candida* colonies (P<0.05, R_s: 0.32).

**Discussion**

In general, the oral health of the HIV positive patients attending the The Department of Pedodontics at the Karolinska Institute was very good. There was no significant difference in the overall oral status of the HIV⁺ group and
the healthy control group. Interestingly however, the children and adolescents with HIV infection exhibited significantly higher *Candida* growth despite ARV treatment.

Oral mucosal lesions are said to be a very common finding amongst HIV infected children (6). Oral candidiasis and herpes simplex infections have been reported at a prevalence ranging from 18-72% and 1.7-24% respectively (31). Contrary to this, no oral mucosal lesions were found amongst the HIV positive children in the present study. This discrepancy is probably related to the treatment given to the infected children in Stockholm, which includes medical therapy and regular check-ups to control virus levels and immune status. All HIV infected patients included in this study, except four children, received medical treatment either in the form of HAART or a single PI. Thus, one might assume that the infected children had an overall good general health and an immunological defense strong enough to suppress the pathogenicity of potentially harmful microorganisms within the oral cavity, such as fungus and herpes simplex viruses. Unfortunately the medical records from the infection clinic were not available when this study was carried out. Information about viral load and immune status was therefore missing. Moreover, it should be emphasized that the medical data and overall health status of the individuals within the control group was obtained by verbal information from the patients or their guardian. No blood tests were taken in order to confirm absence of systemic infections or diseases, which might have affected the results and their reliability negatively.
Previous studies have indicated that HAART improves the oral health of HIV infected individuals (32,33). Conflicting findings have however been reported recently, stating that there is no significant decrease in the prevalence of oral lesions in individuals undergoing HAART compared to those who take other or no ARV treatments (34). HAART has been shown to have a good effect on oral candidiasis as the prevalence of the disease tends to decrease significantly after the initiation of treatment (7,12,13). However, regarding other oral lesions the decrease does not seem to be as significant. This could be due to the fact that the prevalence of many of those lesions already is very low in many industrialized countries, so any significant decrease is hard to demonstrate. (35) Moreover, Flanagan et al. (34) suggested viral resistance to medications, poor compliance with medication therapy and insufficient treatment duration as possible explanations to why one might still find oral lesions in HIV positive individuals receiving ARV treatment. It has been shown that patients receiving HAART for more than 6 months show a significantly lower prevalence of oral lesions (7). The fact that it takes time for the immune system to reconstitute has been suggested as a possible explanation for the previous finding (34). Studies done on healthy and malnourished individuals show that malnutrition (36) and vitamin deficiency (37) could be causing oral candidiasis and should also be taken into consideration if a patient receiving treatment presents with recurrent Candida infection.

Dental caries is said to be a multifactorial disease with carious lesions being a result of interactions between bacteria, the environment (e.g. diet, saliva, Fluoride exposure) and the host (38). Previous studies (14,15,39) on the subject of caries in HIV positive children have concluded that infected
children are more caries active and have a higher caries frequency than non-infected children. Besides advancement of the HIV disease (15), reasons behind increased caries susceptibility amongst HIV infected individuals are mentioned to be medications with high sweetener levels as well as increased feeding frequency and sucrose rich diets in order to compensate for thriving failure (14). Another reason may be painful oral lesions which prevent the maintenance of good oral hygiene (38). Furthermore, Madigan et al. (15) showed that HIV infected children have increased levels of cariogenic bacteria, such as S. Mutans and Lb. In the present study, the majority of the subjects were caries free and the amount of caries active children within the two groups did not differ significantly. No significant difference was seen in the amount of S. Mutans or Lb either. The good dental status could partly be ascribed to the regular dental care the children receive, where large emphasis is put on prophylactic treatment.

Hyposalivation is known to cause a number of problems for the person affected; increased caries risk (40), burning sensation and difficulties with speech and eating are a few examples (41). Some studies (38,42) have shown that patients receiving ARV treatment had a lower salivary flow rate than those who did not take any ARV. In the current study, it was not possible to determine a correlation between medical therapy and stimulated salivary flow amongst the HIV infected children since there were too few individuals who did not receive any medications.

Studies investigating the mean stimulated salivary flow rate amongst children have shown varied results and to date there does not seem to be any
universal consensus on the normal average flow rate. Crossner (25) presented values of stimulated salivary flow rate amongst 805 children (5-15 years old) in Sweden and found a mean of 2.13 (±0.84) ml/min. This value is approximately 1 ml more per minute than the mean salivary flow rate of the children included in the present study. However, a study (26) on 241 Brazilian children between the ages of 6 and 12 years presented a mean salivary flow rate of 1.23 (±0.59) ml/min, which is closer to the values obtained in the current study. Moreover, there was no significant difference in stimulated salivary flow rate amongst the HIV infected children and the controls of the present study, which could suggest that the HIV positive children had a normal flow rate. Björnstad et al. (24) did a comparative study of stimulated salivary flow rates between children from Sweden and Greenland. The mean value differed significantly between the two groups and although it was difficult to determine the reason for this finding, one suggested that masticatory habits, physical activity and climate could be plausible explanations. Furthermore, one should be careful when making comparisons of reference data between different ethnic and cultural groups.

The main difference seen between the two groups in this study was in the amount of Candida colonies found in saliva which was higher within the HIV infected group. Determination of Candida spp. was not carried out, however one would suspect the dominant species to be Candida albicans as it has been reported to be the most common type found in saliva (9,11). Despite the presence of Candida in the saliva, oral candidiasis was not detected in any of the HIV positive children. The 63% who had positive growth of Candida in the saliva were thus asymptomatic carriers. This finding is in line with previous studies (43,44) which have shown that
asymptomatic *Candida* colonization of the oral cavity is a common finding in HIV infected individuals, with prevalence ranging from 28-45%. Furthermore, there seems to be an association between HIV infection and the selection of *Candida albicans* strains with an enhanced ability to adhere to buccal epithelial cells (45,46).

The correlation seen between *Candida* growth in saliva and carious lesions is in agreement with findings of previous studies (9,11). It is debated whether yeast growth in carious lesions is derived from an already ongoing oral candidiasis or if the actual infection is derived from the fungal activity in the decayed teeth. *Candida albicans* can secrete a proteolytic enzyme which facilitates its ability to colonize. These enzymes are able to degrade different types of collagens, such as type 1 which constitutes more than 90% of the organic matrix in dentin. Consequently, its ability to colonize and grow within the carious lesions is enhanced. (11)

To our knowledge, this is the first study that has attempted to identify the level of *Candida* in saliva in healthy children and adolescents in Sweden. Carriage of Candida was analysed in many studies (47,48) using swabs from the oral mucosa or by investigating samples of plaque or carious lesions. In the present study, 34% of the controls were asymptomatic carriers of yeast in saliva. This number is in accordance with the prevalence shown in other studies. In a Polish study including 206 children and adolescents, samples were taken from the pharynx, supragingival plaque and carious lesions. In total, 43.7% of the children were asymptomatic carriers of *Candida albicans* (47). Starr et al. (48) showed a decrease in the prevalence of asymptomatic
Candida carriage when the patients were given regular dental treatment in the form of caries excavation, restorative therapy, dental cleaning and information and instruction in oral hygiene. In the above mentioned study, up to 46% of the children were asymptomatically colonized with Candida at the initiation of the study. The same figure was 27% at the end of treatment, 36 months later. This indicates that good oral hygiene and dental status has an important role in controlling the colonization of Candida in the oral cavity. The prevalence of positive Candida growth increased with age in the control group. The same positive correlation has been shown in a previous study carried out on healthy Swedish children (49). Conversely, within the HIV+ group, the youngest age group (7-9 year-olds) exhibited the highest frequency of positive Candida carriage in saliva. The reason for this discrepancy is unknown. One reasonable explanation could be the fact that many of the younger HIV positive children recently had immigrated to Sweden. In their native countries they had not been receiving ARV treatment and thus had a very poor immune status upon their arrival in Sweden. Most children in the older age groups had been receiving ARV treatment for several years.

Conclusion:
- 34% of the healthy control group was asymptomatic carriers of Candida.
- The HIV infected paediatric patients attending the Department of Pedodontics at the Karolinska Institute between years 2009 and 2011 have an overall good oral health concerning caries and oral lesions.
- Despite an overall good oral health and ARV treatment the HIV positive patients showed a significant higher amount of salivary Candida (63%).
• The clinical significance and implications of elevated levels of *Candida*
  in HIV infected patients needs further investigation.

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