

**DENTURE HYGIENE PRACTICES AND CANDIDA-ASSOCIATED  
DENTURE STOMATITIS AMONG COMPLETE DENTURE  
WEARERS AT TWO CLINICS IN NAIROBI.**

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Dental Surgery (MDS) in Prosthodontics, University of Nairobi.

**BY**

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## DECLARATION

I declare that this thesis is my original work and has not been presented for the award of a degree in any other university.

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## DEFINITION OF TERMS

**Candida - associated denture stomatitis** - Denture stomatitis with positive *Candida* cultures.

**Denture hygiene practices** - Practices carried out by the denture wearer and include denture cleaning method and frequency of cleaning.

**Edentulous** -State of complete lack of teeth.

**Complete dentures** - A set of a maxillary and mandibular denture.

## ACRONYMS

CDS	<i>Candida</i> -associated denture stomatitis
$\chi^2$	Chi square
CFU	Colony forming units
d.f	degree of freedom
DS	Denture stomatitis
DNA	Deoxyribonucleic acid
KNH	Kenyatta National Hospital
PAS	Periodic Acid Schiff
PCR	Polymerase chain reaction
SPSS	Statistical Package for Social Sciences
Type II DM	Type II Diabetes Mellitus
UON	University of Nairobi
UNITID	University of Nairobi Institute of Tropical and Infectious Diseases

## ABSTRACT

**Background:** Studies have shown that one of the most common complications of complete denture wearing is denture stomatitis with a prevalence rate of 11%-75%. Though the aetiology is multifactorial, denture hygiene practices and nocturnal denture wearing have been shown to contribute greatly to the condition.

**Objective:** To determine denture hygiene practices and *Candida*-associated denture stomatitis among complete denture wearers at two clinics in Nairobi.

**Study design:** This was a descriptive cross-sectional study; the study sample comprised of edentulous persons who had been treated at the UON School of Dental Sciences and Kenyatta National hospital and were contacted through telephone and/or post office mail and fulfilled the criteria.

**Materials and method:** An interviewer administered questionnaire was filled with details on denture hygiene practices; denture cleanliness was assessed by soaking the dentures in 5% erythrosine and plaque was graded using the Budtz-Jorgensen criteria of denture cleanliness; denture stomatitis was clinically diagnosed and graded using the modified Newton's criteria by Budtz-Jorgensen and swabs taken from the fitting surface of the denture and palatal mucosa were cultured to assess the *Candida* colony types. A data collection form was used to record the findings for denture cleanliness and *Candida* presence.

**Data analysis and presentation:** The Statistical Package for Social Sciences (SPSS) 12.1 was used for data analysis. Descriptive analytical tests were carried out to determine the frequency of various variables. Pearson's Chi square and independent t-test was applied to test the hypothesis. The data was presented in form of figures and tables.

**Results:** Data of 74 participants was included in the study. Duration of current denture the participants were wearing ranged from 8 months to 24 years with a mean denture age of 6.5 years. The main method of denture cleaning was brushing and soaking in water by 66.2% of the participants while 18.9% soaked in water only, 1.35% soaked in warm salty water and 13.5% brushed only. 51.4% cleaned their dentures once a day, 45.9% more than once while 2.7% cleaned occasionally. Denture cleanliness was excellent in 45.9%, fair in 44.6% and poor in 9.5%. Nocturnal denture wearers were 33.8% and majority cleaned once a day.

Forty five (60.8%) participants had either local 33.8%, diffuse 24.3% or granular 2.7% denture stomatitis. In the cultured specimens, higher Candidal carriage occurred on the denture fitting surface than the palatal mucosa. The predominant species was *C.albicans* followed by *C.krusei*. *C.tropicalis* was co-cultured in two cases from the palatal mucosa. Positive mycological cultures were isolated in higher proportions in participants with the highest grades of inflammation.

**Conclusion:** Within the limitations of this study, majority of the participants reported brushing and soaking their dentures in water and none reported chemical cleansing. Majority cleaned their dentures at least once a day.

The level of cleanliness of the dentures was considered inadequate for half of the denture wearers. A third of the denture wearers reported nocturnal denture wear. Prevalence of DS in this population is 60.8% with only a half of those with DS had positive *Candida* cultures. *C. albicans* was the predominant species and statistically significant associations were observed between denture hygiene

practices and positive *Candida* cultures. Significant association was observed between the denture hygiene practices and denture stomatitis.

**Recommendations:** There is need to establish a health education program aimed at improving denture hygiene practices among complete denture wearers so as to reduce CDS. It is important for clinicians to advice denture wearers on chemical cleansing as mechanical cleansing alone has not been shown to control plaque accumulation on the denture surface.

Clinicians should reinforce avoidance of nocturnal denture wearing since it has been significantly associated with positive *Candida* cultures and other studies need to investigate the other aetiologic factors of DS as only half of those with DS had positive *Candida* cultures.

# CHAPTER 1

## 1.0 INTRODUCTION

Oral health status is influenced by numerous oral diseases and conditions including the loss of teeth and supporting dentoalveolar bone. The prevalence of edentulousness has been reported to range between 6%-29% in non-industrialized countries.<sup>1,2,3</sup> With an increase in the number of people living beyond 60 years of age, which has been estimated to reach 2 billion people by the year 2050 and with 80% of them in developing countries,<sup>4</sup> we are likely to get an increase in demand for complete denture prostheses.

Studies have shown that one of the most common complications of wearing complete dentures is denture stomatitis (DS). This is a common and recurring problem with prevalence rates ranging between 11% and 75%.<sup>5, 6</sup> It is characterized by a lesion that is invariably asymptomatic,<sup>5, 7</sup> usually affects the hard palate and is occasionally associated with angular cheilitis and/or median rhomboid glossitis.<sup>5</sup> DS may lead to soft tissue hyperplasia, negatively affecting denture support. Angular cheilitis may develop which is disfiguring and sometimes painful. The infection can become more severe, even life-threatening, during temporary or permanent immunosuppression.<sup>8</sup>

Risk factors associated with DS are wearing complete in contrast to partial dentures,<sup>9</sup> wearing a maxillary in contrast to a mandibular removable denture,<sup>10</sup> inadequate denture hygiene,<sup>5</sup> nocturnal denture wearing,<sup>6,11</sup> immune

deficiencies and antibiotic therapy,<sup>12</sup> diabetes mellitus,<sup>13</sup> impaired salivary gland function and decreased salivary gland rate,<sup>14</sup> xerogenic medication<sup>15</sup> and gender.<sup>10,12</sup> Studies have shown that many denture wearers do not keep their dentures clean.<sup>10,16</sup> This may be due to lack of preventive hygiene programmes or denture cleanliness awareness.

The aim of this study was to determine the denture hygiene practices and *Candida*-associated denture stomatitis (CDS) in a population of Kenyan complete denture wearers.



## **1.1 LITERATURE REVIEW**

### **1.1.1 Denture stomatitis**

The pathological reactions of the denture-bearing palatal mucosa appear under terms such as denture-induced stomatitis, denture sore mouth, *Candida*-associated denture stomatitis and chronic atrophic candidosis.<sup>17</sup> This is a clinical diagnosis of an inflammatory lesion in which there is redness of the oral mucosa beneath the removable denture.<sup>12</sup> The condition is usually found on the palatal mucosa beneath the fitting surface of the upper denture and it is unusual for it to occur on the lower denture bearing mucosa.<sup>7</sup> Denture stomatitis is frequently asymptomatic, but when signs and symptoms are present they may display mucosal bleeding, swelling, burning or other painful sensations, halitosis, unpleasant taste and drying of the mouth.<sup>7</sup>

### **1.1.2 Classification of denture stomatitis.**

While various classifications have been proposed only a few have found wide clinical acceptance.<sup>7</sup> Newton<sup>18</sup> classified DS into three types. Type I referred to the initial stage of the disease characterized by localized pin-point hyperaemia while Type II is described as having diffuse erythema and oedema of the denture-bearing areas of the palatal mucosa. If type II remains untreated for a long time, Type III develops in which a hyperplastic reaction occurs resulting in a nodular lesion of the central palate referred to as papillary hyperplasia. The view of Thomas<sup>19</sup> on type III differed from Newton<sup>18</sup> stating that papillary hyperplasia may occur in subjects with a papillated mucosa superimposed by denture stomatitis.

Budtz-Jorgensen and Betram<sup>20</sup> classified denture stomatitis according to the type of inflammation observed on the palate under a maxillary denture. There are three types: type I is simple localized inflammation (involving a small area), type II is simple diffuse inflammation involving the whole area covered by the denture and type III is granular inflammation localized to the central part of the hard palate. Type III is a reactive tissue overgrowth, characterized by a hyperemic mucosa with nodular or papillary appearance.

Bergendal and Isacsson<sup>21</sup> modified Newton's classification and used the term local inflammation to describe red spots usually found around the small palatal salivary glands; the lesion was thought to have been associated with trauma from the dentures. They described the second type as diffuse reddening characterized by diffuse hyperaemic, smooth and atrophic mucosa extending over the entire denture area. They described the third type as granulated characterized by hyperaemic mucosa with a nodular appearance in the central part of the palate.

### **1.1.3 Aetiology and predisposing factors.**

Aetiological factors may be divided into two major groups: those related to the prosthesis and those that are infective. Those factors related to the prosthesis include trauma caused by an ill-fitting denture, lack of prosthesis hygiene which is related to the frequency and method of denture cleaning, nocturnal wear, and a favourable environment for proliferation of micro-organisms mainly between the supporting mucosa and the fitting surface of the denture.<sup>5</sup> The infective causes

include some bacteria such as streptococci, staphylococci, *Neisseria* and *Actinomyces* though *Candida* species have most commonly been isolated. <sup>22,23</sup>

Studies have shown that denture wearers are failing to keep their dentures clean. <sup>10,16</sup> Inadequate hygiene of the denture resulting in accumulation of plaque on micro-retention areas on the denture surface is of particular importance as they represent ideal feeding ground for fungi and other microorganisms bringing about the development of infections and inflammation on the mucosa. <sup>24</sup>

Denture hygiene has been reported to be the main means of preventing mucosal inflammation. <sup>25</sup> A study by Pires *et al* <sup>10</sup> showed that 63% of complete denture wearers had poor denture hygiene. These findings were similar to those done by Marchini <sup>16</sup> who conducted a study on 236 complete denture wearers and found that only 27.1% immersed their dentures in a solution. This was attributed to lack of availability and advertising of products for denture care in Brazil and inadequate denture care education. Nocturnal denture wearing has been found to be significantly associated with the prevalence of denture stomatitis. <sup>11</sup> However, other investigators did not find any significant relationship between denture stomatitis and nocturnal denture wearing. <sup>26</sup>

Predisposing factors include tobacco use, xerostomia, <sup>14</sup> high carbohydrate diet, <sup>5</sup> endocrine deficiency, <sup>13</sup> age, <sup>9</sup> nutritional deficiency <sup>6</sup> and prolonged use of drugs such as antibiotics, antifungals and steroids. <sup>12,13</sup>

#### 1.1.4 Virulence factors of *Candida*

The transition of *Candida* from a harmless commensal to a pathogenic organism appears to be dependent on minor changes in predisposing conditions which cause the expression of a variety of virulent factors.<sup>27</sup> These factors include adherence, hyphae formation, thigmotropism, protease secretion, and phenotypic switching phenomenon.

Adherence of micro-organisms is a complex, multifactorial process involving several types of cell surface adhesions which are essential for colonization and infection of the host. The main adhesion molecule of *Candida* responsible for adhesion to host cells seems to be cell wall mannoproteins.<sup>27</sup>

Several other factors also contribute to the adherence of *Candida* such as cell-surface hydrophobicity, environmental pH and concentrations of iron, calcium, zinc and carbon dioxide.<sup>28</sup> Proteins from saliva also affect the complex adherence of *Candida* to host cells and tissues. *Candida albicans* is a pleomorphic micro-organism demonstrating different growth forms such as germ tubes, yeasts (blastospores), pseudo and the hyphae, and chlamydospores.<sup>29, 30</sup>

One of the key virulence determinants of the *Candida* species is their ability to produce and secrete aspartyl protease which digests a variety of host proteins.

The virulence of these proteases has been demonstrated with animal experiments showing that the amount of protease is directly comparable with the pathogenicity of the strain.<sup>31</sup> Therefore, the higher rate of protease activity of

*Candida albicans* in comparison with other *Candida* species also suggests higher virulence. In addition to these virulence factors, *Candida albicans* has a tendency to environmental adaptation. Phenotypic alterations include change of colony morphology and protease activity.<sup>29,32</sup> This genetically controlled phenomenon is known as phenotypic switching and it may occur relatively frequently, especially under stress. Phenotypic switching may assist in the survival of and colonization by the *Candida* in the dentures and it may lead to genetic selection of adaptive strains.<sup>30</sup>

#### **1.1.5 Diagnosis of *Candida*-associated denture stomatitis (CDS).**

Clinical examination of the mucosa overlying the denture fitting surface is the first step in diagnosis. The next step is microbiological identification which is done through swabbing the fitting surface of the denture and palatal mucosa. On the basis of the results a diagnosis can be made. Direct microscopy is a simple and economical approach to detection of the *Candida* species. Like all fungi, the *Candida* species is gram positive and usually can be visualized with a gram stain or Periodic Acid Schiff (PAS).<sup>33</sup> The limitation of direct microscopy is in differentiation of *Candida* species especially *C. albicans* and *C. dubliniensis* which share many morphological and physiological characteristics such as germ tube positivity, biochemical patterns and ability to form chlamydospores in rice and cornmeal extract agar. Hence, the most accurate differentiation method of the 2 species is achieved by performing molecular based techniques such as Polymerase chain reaction (PCR) or Deoxyribonucleic Acid (DNA) fingerprinting.

<sup>34,35</sup> However, these sophisticated techniques are not readily available in routine microbiology laboratories. <sup>35</sup>

### **1.1.6 Isolation methods for *Candida*.**

Clinical specimens from swabs, scraping from the denture, denture sonication or centrifuged saliva can be easily and suitably inoculated on appropriate culture media for isolation of the *Candida* species. The *Candida* species associated with human disease are all able to grow on standard mycological isolation media at 37 degrees. Sabouraud's agar pH5.6 or chromogenic media with Chloramphenicol and Gentamycin added to minimize bacterial contamination is widely used for the culture of *Candida* organisms from clinical samples. <sup>29</sup> The identification of *Candida* organism from clinical samples by biochemical and culture procedures has been reported to take over 24 hours led to a search for more rapid procedures such as DNA fingerprinting <sup>36</sup> which take less than an hour.

## **1.2 RESEARCH PROBLEM**

Tooth loss leading to edentulousness is a global problem. With an increase in the number of edentulous persons, it is likely to translate to more people seeking dentures to restore function and aesthetics. Denture stomatitis has been reported to be a common problem affecting denture wearers.<sup>5,6,7,9</sup> When signs and symptoms are present, they may display mucosal bleeding, swelling, burning or other painful sensations, halitosis, unpleasant taste, dryness in the mouth,

angular cheilitis may develop which is disfiguring and sometimes painful and soft tissue hyperplasia may negatively affect denture support.<sup>9</sup>

*Candida* has been implicated as one of the main aetiological factors in DS.<sup>6</sup> *Candida* colonization of the dentures and underlying mucosa may contribute to the colonization of the gastrointestinal tract and furthermore, oral Candidiasis seems to be a main source of disseminated candidiasis in severely immunocompromized patients.<sup>8</sup> Concerning the emergence of HIV-infection, increase in metabolic diseases such as diabetes and the use of immunosuppressive drugs, interest in infections caused by *Candida* species is on the rise since these are factors that allow the change of a commensal microorganism into a pathogenic one.

### **1.3 JUSTIFICATION OF THE STUDY**

Literature indicates that denture hygiene practices and *Candida* presence influence severity of DS. Information on the situation in developing countries, including Kenya is scarce.

The findings of this study will form a benchmark for further studies which could lead to strategies of prevention of CDS and maintaining oral health for complete denture wearers.

## **1.4 OBJECTIVES**

### **1.4.1 Broad objectives**

To determine denture hygiene practices, cleanliness, nocturnal wear, presence of denture stomatitis and *Candida* among complete denture wearers.

### **1.4.2 Specific objectives**

To determine the denture hygiene practices and level of denture cleanliness among complete denture wearers.

To determine nocturnal denture wearing habits among complete denture wearers.

To determine the prevalence of denture stomatitis among complete denture wearers.

To determine the *Candida* colony types among complete denture wearers.

## **1.5 NULL HYPOTHESIS**

There is no association between nocturnal denture wearing and denture stomatitis among complete denture wearers.

There is no association between frequency of cleaning and denture stomatitis among participants.

There is no association between denture hygiene practices and presence of *Candida* among participants.



## 1.6 VARIABLES

Variable	Measurement
<b>Socio-demographic Variables</b>	
Age of participant	Number of years
Gender	Male or female
Occupation	Type of work done
<b>Independent variables</b>	
Tobacco use	Yes or No
Current denture age	Number of years
Period of denture wearing.	Number of years
Denture cleaning methods	Mechanical and/or soaking in solution
Denture wearing habits	Nocturnal wearer or not
Systemic illnesses	State illness
<b>Dependent variable</b>	
<i>Candida</i>	Colony colour
Denture Stomatitis	Type I Type II Type III

## **CHAPTER 2**

### **MATERIALS AND METHOD**

#### **2.1 Study Design**

This was a descriptive cross-sectional study.

#### **2.2 Study Area**

The study was conducted at the School of Dental Sciences, University of Nairobi and Kenyatta National Hospital.

##### **2.2.1 School of Dental Sciences**

The School of Dental Sciences is situated off Argwings Kodhek road. It offers undergraduate training leading to the Bachelor of Dental Surgery degree and postgraduate training leading to the Master of Dental Surgery (MDS) degrees. This school acts as a referral centre for patients from all parts of the country. Patients are attended to by both undergraduate and postgraduate students under supervision.

### **2.2.2 Kenyatta National Hospital.**

Kenyatta National Hospital (KNH) is the largest referral hospital in Kenya. It is situated off Ngong road in Nairobi. The dental department is situated in the old wing of the hospital. It has divisions catering for all specialities in oral health.

### **2.3 Study population**

The study population comprised of complete denture wearers treated at the undergraduate prosthetic clinic and KNH prosthetic clinic between 2002 and 2008.

### **2.4 Sample size determination.**

The sample size was calculated using Fisher's method of sample size determination and based on a study by Kulak-Ozkan<sup>26</sup> which reported that the prevalence of denture stomatitis was 44%.

Hence:-

$$N = z^2 pq / d^2$$

Where,

N=the desired sample size (when population is greater than 10000)

z= standard normal deviate, usually set at 1.96 which corresponds to the 95% confidence level.

p=prevalence

q=1.0-p

d=degree of accuracy desired set at .05

Using the formula:

$$N=z^2pq/d^2$$

$$N= (1.96)^2(0.44) (0.56)/ (0.05)^2$$

$$=368$$

Records from the UON dental school clinic showed the total number of denture wearers seen since 2002 to 2008 were 924 but those who were available were 90. Considering only those available, the final sample estimate ( $n_f$ ) was calculated using the following formula.

$$n_f=N/1+ (N/n)$$

Where;

$n_f$ =the desired sample size when the population is less than 10000.

$N$ =the desired sample size when the population is greater than 10000.

$n$ =the estimate of the population size.

Hence;

$$n_f=368/ (1+368/90)$$

The minimum sample size was calculated to be 73. However, 74 participants were involved in the study.

## **2.5 Sampling procedure**

Purposive sampling was done. Records of 924 complete denture wearers were available but only 427 had post office or telephone contacts. Out of 427 contacted, only 90 responded. Sixteen did not fulfil the inclusion criteria.

### **2.5.1 Inclusion criteria**

Participants with complete dentures.

Complete denture wearers aged 18 years and above.

Participants who had worn dentures for more than six months.

### **2.5.2 Exclusion criteria**

Participants who have been on corticosteroids, antibiotics for more than two weeks or antifungals within the last 3 months.

Participants whose ages were below 18 years.

Single denture wearers.

Those who were not wearing their dentures.

Those who had used their dentures for less than six months.

## **2.6 Data collection instruments and technique.**

### **2.6.1 Questionnaire.**

An interviewer administered semi-structured questionnaire with socio-demographic data (age, gender, occupation) denture cleaning methods, denture wearing habits, tobacco use and systemic illnesses was recorded immediately before the clinical examination.

The denture cleaning methods were either mechanical and/or chemical cleansing. Mechanical cleansing was determined through the question on brushing while chemical cleansing was determined through the question of solution used to soak the dentures in. Soaking of dentures involved immersing them in a liquid for not less than one hour.

## **2.6.2 Data collection form.**

### **2.6.2.1 Clinical examination**

Clinical examination was done in a clinical setting. The principal investigator did the examination under similar lighting with the patient lying supine on a dental chair. Strict hygienic standards were maintained with use of gloves and dental examination mirrors. The degree of palatal erythema was scored using the classification of denture stomatitis by Budtz-Jorgensen<sup>20</sup> as it describes the inflammation seen on the palatal mucosa. Information obtained was entered in the data collection form.

0: No inflammation

I: Slight inflammation (localized/pin point hyperaemia)

II: Moderate inflammation (diffuse hyperaemia)

III. Diffuse hyperaemia with inflammatory papillary hyperplasia.

In addition, presence of angular cheilitis and median rhomboid glossitis were assessed and findings recorded in the data collection form.

### **2.6.2.2 Denture cleanliness.**

Denture cleanliness was assessed using a plaque detector (5% Erythrosine) to disclose the plaque on fitting surface of the maxillary denture. The maxillary complete denture was rinsed to remove food debris for two minutes before using the plaque detector. The dentures were soaked for five minutes in the plaque detector and according to the quantity of the plaque on the denture base; participants were sub-divided into three groups using Budtz-Jorgensen Index<sup>20</sup> of denture cleanliness.

Excellent: None or few spots of plaque.

Fair: More extended plaque less than half the denture base covered by plaque.

Poor: more than half the denture base covered by plaque.

### **2.6.2.3 Mycological Examination**

The palatal mucosa and the fitting surface of the denture were swabbed with sterile cotton swabs. The swabs were transported to the University of Nairobi Institute of Tropical and Infectious Diseases (UNITID) within 1 hour of taking the swab and streaked on the culture media. They were incubated at 37 degrees Celsius for 48 hours in ChromID media and different species were identified according to the colour of the colonies cultured.

## **2.7 Data validity and reliability**

The study population was from the Prosthetics dental clinic at UON School of Dental Sciences and Kenyatta National Hospital Prosthetics Department. Pretesting of the data collection instruments was done and corrections made. The principal investigator was calibrated by the supervisors to calculate inter-examiner reliability in diagnosis and classification of DS, method of collecting swabs for culture and

assessment of the cleaning of the denture. Cohen's Kappa was used to calculate intra-examiner reliability and a value of 0.93 achieved.

## **2.8 Data analysis and presentation.**

The data collection forms were pre-coded. The data collected was analyzed using the statistical package for social sciences (SPSS) 12.1 (SPSS Inc, Chicago, Illinois, USA). Calculations were done to determine frequencies of the various variables. The information obtained was organized and presented as descriptive statistics in form of frequency tables and bar graphs. Correlating various variables was done using appropriate statistics and a P value of 0.05 or less was considered statistically significant. The Student t-test was used to compare the mean ages between the gender and between the duration of denture wearing and current denture use among those with positive and negative *Candida* cultures. Pearson Chi-square test was used to determine the relationship between various categorical variables.

## **2.9 Control of biases and errors**

Errors in data collection were minimized by standardization of the examination to control for intra-examiner errors. This was done using a pilot survey on a few participants to check for consistency of the examiners. This helped reduce inter-examiner variability. All data collection tools were pre-tested and the incubator was calibrated for temperature. The principal investigator was calibrated by the supervisors



to calculate inter-examiner reliability in diagnosis and classification of DS, method of collecting swabs for culture and assessment of the cleaning of the denture. Cohen's Kappa was used to calculate intra-examiner reliability and a value of 0.93, 0.97 and 0.91 was obtained respectively.

## **2.10 Ethical consideration**

The proposal was submitted to the Kenyatta National Hospital and University of Nairobi Ethics, Research and Standards Committee for approval.

The purposes of the study and expected benefits were clearly explained to the participants and signed consent was obtained from them. Information obtained was kept confidential. Participation was voluntary and participants were at liberty to terminate participation without victimization and no treatment was denied on termination. The entire examination was carried out maintaining strict hygienic standards and those requiring treatment were referred.

## **2.11 Perceived benefits**

The study will provide a relevant reference point to the clinicians in the clinical practice particularly in management of denture wearers and can also be used by clinicians to educate the patient on denture hygiene practices and denture cleanliness. The findings will add to the existing literature on the African prevalence rates of denture stomatitis and will act

as a benchmark from which other studies can further delve into more specific areas of research on *Candida*-associated denture stomatitis.

The study will also serve as a partial fulfilment on the Master of Dental Surgery degree in Prosthodontics, University of Nairobi.

## **2.12 Limitations to the study.**

1. Only those with phone numbers and/or postal addresses were contacted hence data may not be representative of the whole sample.
2. Culture medium selected could not identify *Candida glabrata*.

## CHAPTER 3

### RESULTS

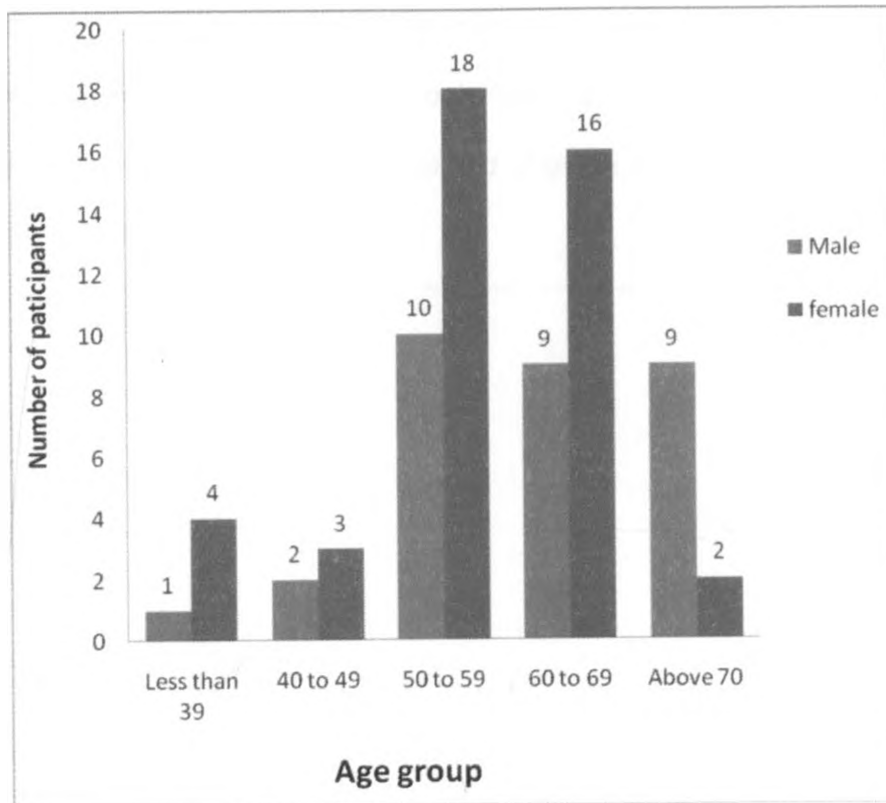
#### 3.1 Sociodemographic characteristics.

A total of 74 patients were included in the study. Of these, 43(58.11 %) were females while 31(41.89 %) were males. Their ages ranged between 29 and 77 years with a mean age of  $59 \pm 9.92$  SD (Figure 1). The females were younger (mean age  $59 \pm 9.70$  SD) than the males (mean age  $62 \pm 9.61$ ) and the difference was statistically significant ( $t=2.23$  d.f 72,  $p=0.029$ ).

Denture wearing history was between 8 months to 27 years with a mean age of 6.5 years while the range for current denture worn was between 8 months and 24 years with a mean age of 3.97 years.

Majority of the dentures had been worn for 5 years or less, 54(73%); 15(20%) had used their current dentures for 6-9 years while 5(7%) had used for 10 years and longer. Few participants 17(22.97%) reported presence of a systemic disease with 5(6.76%) reporting type II diabetes mellitus.

Ten (13.5%) participants reported tobacco use and of these, nine (90%) were males.



**Figure 1: Distribution of participants according to gender and age**

### **3.2 Denture hygiene practices**

#### **3.2.1 Denture cleaning methods.**

None of the participants reported use of chemical cleansers or palatal brushing. The majority of the participants 49(66.22%) reported brushing and soaking in water, 14(18.92%) reported soaking in water only, 10(13.5%) reported brushing their dentures only while 1(1.35%) soaked in warm salty water (Figure 2).

Majority of the females 32(74.4%) reported brushing and soaking their dentures, 6(14%) soaked in water only, 4(9.3%) reported brushing only while 1(2.3%) soaked in warm salty water. This was in contrast to the

males who 17(54.8%); 8(25.8%) and 6(19.4%) reported brushing and soaking; soaking and brushing only respectively though no association was seen between gender and method of cleaning. (Pearson Chi  $\chi^2 = 3.20$ , df 2, p=0.202)

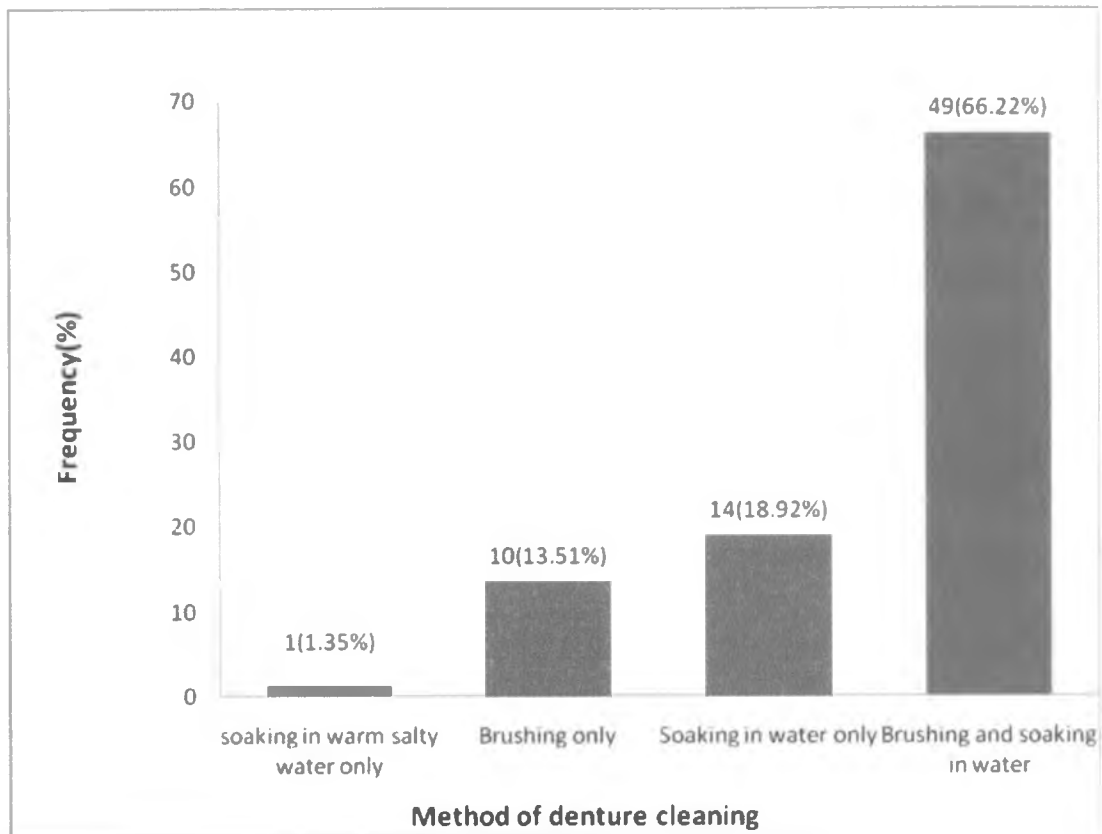


Figure 2. Distribution of denture cleaning methods.

### 3.2.2 Frequency of denture cleaning.

Majority of the participants cleaned once a day 38(51.4%), 34(45.9) more than once and 2(2.7%) occasionally. The females participants cleaned their dentures more than once 32(74.4%) while 11(25.6%) cleaned once a day. Majority of the males 27(87%) cleaned once a day, while an equal number cleaned occasionally 2(6.5%) and more than once 2(6.5%). There was a

significant association between frequency of cleaning and gender (Chi  $\chi^2$  with Yates correction =30.82, df 1, p=0.000).

### 3.3 Denture cleanliness.

Using the Budtz-Jorgensen scale of denture cleanliness, 34(45.9%) participants' dentures were found to be excellent, 33(44.6%) were found to be fair and 7(9.5%) were found to be poor. When males were considered, 6(19.4%) had excellent denture hygiene, 21(67.7%) had fair and 4(12.9%) had poor cleanliness. Majority of the females 27(62.8%) had excellent denture cleanliness, 12(27.9%) had fair and 4(9.3%) had poor denture cleanliness respectively showing that the female participants had cleaner dentures than the males (Table 1) and this was statistically significant ( $\chi^2$  with Yates correction =13.40, df 1, p=0.000).

**Table 1: Association between denture cleanliness and gender.**

Denture Cleanliness	n	%	Male	Female	Test statistics
Excellent	34	45.9	6(19.4%)	27(62.8%)	$\chi^2=13.40$ , df 1, p=0.000
Fair	33	44.6	21(67.7%)	12(27.9%)	
Poor	7	9.5	4(12.9%)	4(9.3%)	
<b>Total</b>	74	100	31	43	

### **3.4 Nocturnal denture wearers.**

Twenty five (33.8%) participants reported to wearing their dentures at night while 49(66.2%) removed them at night. Of the males 13(41.9%) were nocturnal wearers while fewer females, 12(27.9%) reported nocturnal wear though there was no significant association between the gender and nocturnal denture wearing (Pearson Chi  $\chi^2$  with Yates correction =1.02, df 1, p=0.225).

Majority of those who cleaned their dentures once a day practiced nocturnal denture wearing 18(72%) while of those who did not practice nocturnal wear, 22(55.10%) cleaned more than once a day. There was a significant association between nocturnal denture wearing and frequency of cleaning (Pearson's  $\chi^2$  with Yates correction =3.87, df 1, p=0.049).

### **3.5 Denture stomatitis.**

A total of 29(39.2%) participants did not have any signs of denture stomatitis whereas 45(60.8%) had either local 25(33.8%), diffuse 18 (24.3%) or granular 2(2.7%) stomatitis. None of the participants had either angular cheilitis or median rhomboid glossitis.

Denture stomatitis was seen more often among the males 25(55.5%) compared to the females 20(44.5%) and there was a significant association between gender and DS (Pearson Chi  $\chi^2$  with Yates continuity correction =7.43, df 1, p=0.006).

### 3.6 Distribution of *Candida* species on the palatal mucosa and fitting surface of the dentures.

The prevalence of positive cultures was 29(39.20%) and 25(33.78%) on the fitting surface of the denture and palatal mucosa respectively (Table 2). The distribution of *C.albicans*, *C.krusei* and both *C.albicans* and *C.krusei* on the fitting surface of the denture and palatal mucosa was as shown on table 2.

**Table 2: Distribution of *Candida* species on the palatal mucosa and fitting surface of the dentures.**

CANDIDA SPECIES	DENTURE	PALATE
<i>C.albicans</i>	17(58.62%)	15(60%)
<i>C.krusei</i>	6(20.69%)	6(24%)
<i>C.albicans</i> and <i>C.krusei</i>	6(20.69%)	2(8%)
<i>C.albicans</i> , <i>C.krusei</i> and <i>C.tropicalis</i> .	0	2(8%)
Total	29(39.20%)	25(33.78%)

#### 3.6.1 Distribution of *Candida* isolated from the cultures of fitting surface of dentures by clinical indexes.

In the cultures from the fitting surface of the denture, no growth was detected in 45(60.81%). Of these 45(60.81%) cultures from the fitting surface of dentures, 27(60%) belonged to the participants without denture stomatitis (type 0) and 18(40%) belonged to those with denture stomatitis



(seventeen from type I, and 1 from type II). The distribution of the different *Candida* species cultured according to type of DS is shown on table 3.

**Table 3: Distribution of *Candida* isolated from the cultures of the fitting surface of dentures by clinical indexes.**

Type of DS	Gender	<i>C.albicans</i>	<i>krusei</i>	<i>C.albicans</i> + <i>C.krusei</i>	Culture negative
Type 0 (n=29)	Female(n=23)	2	0	0	21
	Male(n=6)	0	0	0	6
Type I (n=25)	Female(n=13)	3	1	0	9
	Male(n=12)	3	1	0	8
Type II (n=18)	Female(n=5)	4	1	0	0
	Male(n=13)	5	3	4	1
Type III (n=2)	Female(n=2)	0	0	2	0
Total	74	17	6	6	45

### 3.6.2 Distribution of *Candida* isolated from the cultures of the palatal mucosa by clinical indexes.

In the swabs taken from the palatal mucosa of 29(39.19%) without denture stomatitis (type 0), negative cultures were seen in 27(93.1%). With regard to 45(60.81%) with denture stomatitis, culture results were positive in 23 (51.1%).

The prime culture species isolated from the palatal mucosa was *C.albicans* in 19(25.68%). In 15(20.28%) of the cultures, *C.albicans* was isolated as pure culture (without any other species) while it was isolated

together with other species in 4(5.4%) cultures. *C.krusei* was the second most common species isolated in 6(8.10%) cultures and mixed cultures were observed in 4(5.4%). A mixed species of *C. albicans*, *C.krusei* and *C. tropicalis* was observed in 2(2.7%) cases and only occurred on the palate (Table 4).

**Table 4: Distribution of *Candida* isolated from the cultures of the palatal mucosa by clinical indexes.**

Type of DS	Gender	<i>C.albicans</i>	<i>C.krusei</i>	<i>C.albicans</i> <i>+krusei</i>	<i>C.albicans</i> <i>+krusei</i> <i>C.tropicalis</i>	Culture negative
Type 0 (n=29)	Female(n=23)	2	0	0	0	21
	Male(n=6)	0	0	0	0	6
Type I (n=25)	Female(n=13)	2	1	0	0	10
	Male(n=12)	3	1	0	0	8
Type II (n=18)	Female(n=5)	4	1	0	0	0
	Male(n=13)	4	3	0	2	4
Type III (n=2)	Female(n=2)	0	0	2	0	0
Total	74	15	6	2	2	49

### **3.7 Mean age of dentures and denture wearing among wearers.**

The mean age of the current dentures the participants were wearing was 4 years. The dentures from which *Candida* was cultured had been worn for a mean age of 6.71 years while those with negative *Candida* cultures had a mean age of 2.20 years. The number of years the participants had worn their dentures ranged from 8 months to 27 years with a mean of 6.5 years. Of those with positive *Candida* cultures on either the palatal mucosa or the fitting surface of the denture, the mean age was 10.79 years compared to 3.73 years for those whose cultures were negative. There was a statistically significant difference in the duration of wear, age of current dentures and presence of *Candida*. ( $t= 4.86$ , d.f 42.33 and  $p=0.000$ ;  $t= 4.14$  d.f, 31.27 and  $p=0.000$  respectively)

### **3.8 Association between frequency of cleaning, denture cleanliness, nocturnal denture wearing and DS.**

Majority of those with type II and III DS cleaned once in a day 17(85%) while majority of those without DS cleaned more than once in a day (table 5) and this was statistically significant (Pearson Chi  $\chi^2$  with Yates correction =19.16 , df 2,  $p=0.000$ ). Severity of DS increased with nocturnal denture wearing and fair/poor denture cleanliness and these were statistically significant (  $\chi^2 =28.84$ , df 2, $p=0.000$ ;  $\chi^2 =44.32$ , df 2, $p=0.000$  respectively).

Therefore, the null hypothesis that nocturnal denture wearing and denture cleanliness have no association with DS is rejected.

**Table 5: Association between frequency of cleaning, nocturnal denture wearing, denture cleanliness, *Candida* and DS.**

		Type 0	Type I	Type II and III	Test statistic
Frequency Of cleaning	Occasionally	0	0	2	$\chi^2 = 19.16$ , df 2, $p=0.000(<0.05)$
	Once a day	7	14	17	
	More than once a day	22	9	3	
Nocturnal denture wearing	Yes	2	7	16	$\chi^2 = 28.84$ , df 2, $p=0.000(<0.05)$
	No	27	18	4	
Denture cleanliness	Excellent	27	6	1	$\chi^2 = 44.32$ , df 2, $p=0.000(<0.05)$
	Fair/Poor	2	19	19	
Presence of <i>Candida</i>	Absent	27	16	2	$\chi^2 = 34.47$ , df 2, $p=0.000(<0.05)$
	Present	2	9	18	

### **3.9 Association between denture hygiene practices, denture cleanliness, nocturnal denture wearing and *Candida* presence.**

Majority of those who brushed and soaked their dentures 40(88.9%) had no *Candida* growth and there was a significant association between presence of *Candida* and cleaning method. (Pearson's Chi  $\chi^2=28.46$ , df 2,  $p=0.000$ ). Positive cultures were seen in 37(93.1%) of those with fair and poor denture cleanliness and a significant association was observed between denture cleanliness and presence of *Candida* (Pearson's Chi  $\chi^2=26.75$ , df 1,  $p=0.000$ ).

Those who cleaned once or occasionally, majority 32(79.31%) had positive *Candida* cultures compared to those who cleaned more than once a day 7(20.69%) and this was statistically significant (Pearson's Chi  $\chi^2=10.63$ , df 1,  $p=0.001$ ).

Twenty two (75.86%) nocturnal denture wearers had positive cultures compared to 7(24.14%) who did not practice nocturnal denture wear and a significant association was noted (Pearson's Chi  $\chi^2=34.72$ , df 1,  $p=0.000$ ). Therefore, the null hypothesis that denture hygiene practices have no association with *Candida* is rejected.

## CHAPTER 4

### DISCUSSION

#### 4.1 Sociodemographic variables

The mean age of the participants was 59 years which was lower than that reported in other similar studies done in Asia,<sup>37</sup> Jordan,<sup>38</sup> Brazil<sup>10, 39, 40, 41</sup> and the UK.<sup>42</sup> The difference may be due to the sampling technique used for this study as this group of denture wearers either had mobile phones or postal addresses compared to an older population of denture wearers who may not have these facilities. Also, the older population of denture wearers may have used phone numbers and addresses of their relatives who may not have seen the need to bring them to the clinics for the study limiting the number of those available.

#### 4.2 Frequency and method of denture cleaning

In the population studied, 97.3% stated that they cleaned their dentures daily. These results agree with Luciano *et al*<sup>40</sup> (98%), Marchini *et al*<sup>16</sup> (98.7%) and Nevalainen *et al*<sup>43</sup>(96%) but are higher than that reported by Hoad-Reddick<sup>44</sup> who showed that only 79% of a sample of 233 participants cleaned their dentures daily. Grant *et al*<sup>45</sup> demonstrated that there is a strong correlation between unsatisfactory denture cleanliness and frequency of cleaning. However, according to Nevalainen *et al*,<sup>43</sup> this

frequency does not necessarily indicate efficient cleaning, mainly because 46.7% of the sample they studied were 80 years and above with limitations such as reduction in visual acuity and manual dexterity.

On the method of denture cleaning, majority brushed their dentures and soaked them in water. None of them added any chemicals to the water. It is important to find out why chemical cleansing products were not used as mechanical cleansing alone may not be adequate to eliminate plaque on the denture. Ideally, both mechanical and chemical cleansing should be used together to achieve better plaque control.<sup>37</sup>

#### **4.3 Nocturnal denture wearers.**

The present study revealed that 33.8% of the participants reported nocturnal denture wearing. The prevalence of nocturnal denture wearers is lower than that reported by Luciano *et al*<sup>40</sup> and Coco *et al*<sup>42</sup> (64% and 67% respectively). This difference may be a reflection of the younger sample population used in this study unlike that of Coco *et al*<sup>42</sup> who dealt with the elderly with a mean age of 73 years while that of Luciano *et al*,<sup>40</sup> the participants had a mean age of 67.3 years.<sup>42</sup>

#### **4.4 Denture stomatitis**

The results of the present study showed a prevalence of 60.8% as has been reported in some studies<sup>10, 39</sup> though others have reported lower prevalence rates.<sup>12, 38</sup> The variability between different investigations can

be explained by the subjectivity of the classification, and thus several authors have suggested using different indices.<sup>5,6,21</sup> The most popular methods of classification for denture stomatitis are derived from Newton's<sup>18</sup> original method that scores according to the severity of the erythema. Although Newton's classification is useful for characterizing the severity of stomatitis, its limitations are the implication that type III DS develops when type II DS remains untreated for a long time and when researchers attempt to establish a link of DS with microbiologic findings.<sup>5</sup> It has been shown that type II DS does not necessarily progress to type III DS<sup>19</sup> hence Budtz-Jorgensen's classification is considered a gold standard in the classification as it describes the type of inflammation observed.<sup>20</sup>

In the present study, type I DS was most common; we found 25 patients with type I (33.8%), 18 with type II (24.3%) and 2 with type III (2.7%). Only Kulak-Ozkan *et al*<sup>29</sup> and Pires *et al*<sup>13</sup> showed a prevalence of type I higher than this study (51.7% and 53.8%). This may be due to different calculation methods used as they only considered those with denture stomatitis while this study based its calculations on the whole sample population including those without denture stomatitis.

There was a higher prevalence of DS in males compared to females and this was statistically significant. This has been reported in previous reports<sup>41,46,47</sup> but others have shown equal or more DS in females compared to the males.<sup>5,10,33,48</sup> Perhaps because majority of the males were nocturnal



denture wearers or they had a higher tolerance to mucosal changes may explain why the prevalence was higher in males.

In this study, the prosthetic variables that were significantly associated with DS were denture cleanliness, frequency of cleaning and nocturnal wearing. Other authors have found similar variables related with the prosthesis to be significant in the development of DS.<sup>6,10,49,50</sup> This is in contrast to Figueiral *et al*<sup>5</sup> and Tarbet *et al*<sup>51</sup> who found no correlation between denture cleanliness and DS and this may be related to the index used to evaluate the cleanliness of the prosthesis. According to Helena *et al*<sup>52</sup>, weak inter-instrument reproducibility with other indices except the Budtz-Jorgensen index<sup>20</sup> was observed on assessment of denture cleanliness. Therefore, this should be the method of choice for clinical studies when more sophisticated approaches like computerized methods are not possible.

#### **4.5 *Candida* and other risk factors.**

In this study, 9 out of 10 tobacco users had positive *Candida* cultures which is similar to that reported by other authors.<sup>6, 53</sup> The exact mechanism by which candidal carriage may be affected by tobacco or cigarette smoke is not yet established.<sup>53</sup> However, it has been suggested that smoking and friction caused by the denture to the oral mucosa may facilitate candidal colonization. Alternatively, cigarette smoking may contain nutritional factors for *Candida*.<sup>6, 54</sup>

In this study, the prosthetic variables that were significantly associated with *Candida* species were denture cleanliness, frequency of cleaning, age of dental prosthesis, number of years of denture wearing and nocturnal wear. These factors have been studied before and results are similar<sup>26,37,38</sup> though Ryu *et al*<sup>55</sup> did not show any relationship between age of denture, denture cleanliness and presence of *Candida*. This difference may be due to differences in methodology as Ryu *et al*<sup>55</sup> had a cut off point indicating *Candida* CFU levels that were considered significant.

Positive *Candida* cultures were observed in 88% of the nocturnal denture wearers. This has been observed in other studies.<sup>5, 12, 14</sup> This may be explained by the presence of dentures overnight protecting the micro-organisms from removal by swallowing and retaining nutrients in environmental conditions favourable to *Candida*.<sup>12, 14, 56</sup>

*C. albicans* was the predominant species in the cultures obtained from both palatal mucosa and fitting surface of dentures. These results are similar to what has been reported in literature.<sup>23,41,42,57,58</sup> *C. krusei* was the second highest cultured species which contrasts with what has been reported in other studies.<sup>41,42,57,58</sup> Saadatin *et al*,<sup>41</sup> Coco *et al*<sup>42</sup> and Rafael *et al*<sup>58</sup> used ChromID*Candida*<sup>(R)</sup> which identified 5 different species of *Candida* and found *C. glabrata* as the second highest while ChromeAgar<sup>(R)</sup> identifies 3 species.

When positive cultures were evaluated according to type of DS, higher levels of *Candida* were observed as denture stomatitis intensified as has also been reported by Saadatin *et al.*<sup>41</sup> This may indicate a possible causal role of *Candida* in denture stomatitis.

## CONCLUSION

Within the limitations of this study, we can conclude that:

1. Majority of the participants reported brushing and soaking their dentures in water and none reported chemical cleansing with 98% cleaning one or more times in a day.
2. The level of cleanliness of the dentures was considered inadequate among half of the denture wearers.
3. A third of the denture wearers reported nocturnal denture wear.
4. Prevalence of DS in this population is 60.8% (Type I, 33.8%; Type II, 24.3% and Type III, 2.7%) and only a half of those with DS had positive *Candida* cultures.
5. *C. albicans* was the predominant species and statistically significant associations were observed between denture hygiene practices and positive *Candida* cultures.
6. Significant association was observed between the denture hygiene practices and denture stomatitis.

## RECOMMENDATIONS

There is need to establish a health education program aimed at improving denture hygiene practices among complete denture wearers so as to reduce CDS.

It is important for clinicians to advice denture wearers on chemical cleansing as mechanical cleansing alone has not been shown to control plaque accumulation on the denture surface.

Clinicians should reinforce avoidance of nocturnal denture wearing since it has been significantly associated with positive *Candida* cultures.

Other studies need to investigate the other aetiologic factors of DS as only half of those with DS had positive *Candida* cultures.

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# APPENDICES

## APPENDIX I: CONSENT FORM.

I..... do hereby consent to take part in this study on denture hygiene practices and denture stomatitis among complete denture users. I understand that no invasive procedures are to be carried out to cause psychological or bodily harm and my participation is voluntary. In case of any pertinent findings, I will be given advice regarding the condition and will be referred for relevant management. I understand that all the information I give and the clinical findings will be treated with strict confidentiality and will be used for the sole purpose of research.

Signature of participant.....

Signature of investigator.....

## APPENDIX II: QUESTIONNAIRE

### Questionnaire

- (1) Demographic data
- (a) Age (years)
  - (b) Gender      Female       Male
  - (c) Occupation
- (2) Denture status
- (a) Years of denture wearing .....
  - (b) Age of denture .....
- (3) Systemic illness .....
- (4) Denture cleaning methods
- (a) Brushing only
  - (b) Soaking in solution only
  - (c) Soaking in water only
  - (d) Brushing and soaking in solution
  - (d) Brushing and soaking in water
  - (e) Nothing
  - (ii) Solution used (state)
- (5) Cleaning frequency
- ( a )Occasionally
  - ( b) Once a day
  - ( c )More than once a day
- (6) Palatal brushing      Yes       No
- (7) Denture wearing habits

Nocturnal wearer

Yes

No

(8) Tobacco use.

Yes

No

(9) How often do you visit the dentist? .....

(a) Rarely (Less than once a year after the last control visit)

(b) Occasionally (at least once a year since the last control visit)

(c) Never (since the last control visit)

## APPENDIX III: DATA COLLECTION FORM

PATIENT NAME

### CLINICAL EXAMINATION

The degree of palatal erythema will be scored using the modified Newton's classification of denture stomatitis by Burtz-Jorgensen (1970).

0: No inflammation

I: Slight inflammation (Localized/pin point hyperaemia)

II: Moderate inflammation (Diffuse hyperaemia)

III: Diffuse hyperaemia with inflammatory papillary hyperplasia

Median Rhomboid glossitis

Present

Absent

Angular cheilitis

Present

Absent

### DENTURE CLEANLINESS

1.Excellent

2. Fair

3.Poor





APPENDIX IV

KENYATTA NATIONAL HOSPITAL  
Hospital Rd. along, Ngong Rd.  
P.O. Box 20723, Nairobi.  
Tel: 726300-9  
Fax: 725272  
Telegrams: MEDSUP\*, Nairobi.  
Email: [KNHolan@Ken.Healthnet.org](mailto:KNHolan@Ken.Healthnet.org)  
24<sup>th</sup> December 2008

Ref: KNH/UON-ERC/ A/131

Dr. Margaret Ndung'u-Mwasha  
Dept. of Conservative and  
Prosthetic Dentistry  
School of Dental Sciences  
University of Nairobi

Dear Dr. Ndung'u

**RESEARCH PROPOSAL: "QUALITY OF DENTURE HYGIENE AND CANDIDA-ASSOCIATED  
DENTURE STOMATITIS AMONG COMPLETE DENTURE WEARERS AT TWO CLINICS IN NAIROBI"  
(P283/10/2008)**

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This is to inform you that the Kenyatta National Hospital Ethics and Research Committee has reviewed and **approved** your above revised research proposal for the period 24<sup>th</sup> December 2008 -23<sup>rd</sup> December 2009.

You will be required to request for a renewal of the approval if you intend to continue with the study beyond the deadline given. Clearance for export of biological specimen must also be obtained from KNH-ERC for each batch.

On behalf of the Committee, I wish you fruitful research and look forward to receiving a summary of the research findings upon completion of the study.

This information will form part of database that will be consulted in future when processing related research study so as to minimize chances of study duplication.

Yours sincerely

PROF. A. N. GUANTAI

**SECRETARY, KNH/UON-ERC**

c.c. Prof. K.M. Bhatt, Chairperson, KNH-ERC  
The Deputy Director CS, KNH  
The Dean, School of Dental Sciences, UON

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