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The Effect Of Cognitive Status And Residency On Oral Health In Patients With Dementia

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THE EFFECT OF COGNITIVE STATUS AND RESIDENCY ON ORAL HEALTH IN PATIENTS WITH DEMENTIA

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A

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ABSTRACT

The effect of cognitive status and residency on the oral health of patients with dementia

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Poor oral health is a predictor of cognitive decline in elderly populations and has been shown to precede dementia. As cognitive decline progresses, patients are likely to move from the community into nursing facilities. We hypothesize that severity of dementia and residency type will impact the oral health of patients with dementia. Fifty-two participants of two dementia levels were recruited from the UTHealth Neurocognitive Disorders Center and two Houston-area nursing homes. A standardized oral health assessment, plaque index, and oral bacteria analysis determined participants' oral health status. Further, data was collected on participants' medical history, oral hygiene habits, dietary habits, and swallowing ability. Across dementia level, we found no visible differences in oral health, but we did find microscopic differences in oral bacterial composition between patients with mild and severe dementia. Of the 127 species that significantly differed, bacteria causing periodontitis, tooth decay, and pneumonia were found in greater abundance in patients with severe dementia. Further, patients with severe dementia had significantly worse swallowing ability, which can result in fatal aspiration pneumonia. Across residency type we found that compared to community dwelling patients with dementia, nursing home residents with dementia have significantly worse oral health according to the number of teeth chewing pairs, plaque index, and oral bacteria composition. Of the 138 species that significantly differed across residency type, we found nursing home subjects had greater abundance of oral disease-causing bacteria. Overall, we recommend that oral health assessments of patients with dementia not only include a visual oral

health screening, but also include an analysis of oral bacteria composition as pathogenic oral species have grave potential to worsen oral and systemic disease.

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CHAPTER 1: INTRODUCTION

Dementia

Dementia is a neurocognitive disorder that causes memory loss, learning impairment, affects executive and language function, and is a disorder for which research has yet to determine a clear mechanism or cure. About 55 million people are currently living with dementia; this number is expected to double every 20 years (World health organization (WHO, 2021). The high prevalence of this disease worldwide resulted in an annual global cost of $1.9 trillion, the GDP of the country Italy (WHO, 2021; datacommons.org / World bank). The growing prevalence of the disease and increasing costs of healthcare has caused the WHO to estimate a global cost of $2.8 trillion by 2030 (WHO, 2021). With a growing prevalence and financial burden of dementia, it is imperative research focuses on discovering risk factors, earlier diagnostic methods, and better treatments for this disorder.

Dementia is most common in elderly individuals, with age being the strongest risk factor for dementia. Other risk factors include family history, genetics, gender, race, smoking history, excessive alcohol use, head trauma, and comorbidities such as diabetes, cardiovascular disease, and hypertension (Flier, 2005; Gill, 2020; Livingston, 2020). About 55% of patients living with dementia are in a mild stage of the disease, 32% in a moderate stage, and about 12% of patients living with dementia are in a severe stage of the disease (Prince, 2014). Due to increased impairment and interference with everyday living activities, as the disease progresses, individuals are more likely to require and use long term care in the form of a caregiver or nursing facilities (Mather, 2020).

There are many subtypes of dementia. Dementia can be caused by multiple illnesses, with Alzheimer's Disease (AD) being the most common dementia-causing illness. Other disorders and diseases that cause dementia include strokes, Lewy-Body Dementia (LBD), and Frontotemporal Dementia (FTD) (Holtzman, 2012). A study of 382 subjects with dementia found that 77% of participants had AD, 26% had LBD, 18% had Vascular Dementia (VaD), 13% had Hippocampal Sclerosis (HS), and 5% had FTD. AD was also present in a majority of participants with LBD, VaD, and HS (Barker, 2002). Though there are many diseases that cause dementia, all dementias share a common mechanism of neuroinflammation responsible for disease onset and progression (Raz, 2016). The leading cause of death in individuals with dementia is pneumonia, which accounts for about 38.4% of death in patients with dementia. This percentage is significantly greater than the 2.8% of the general elderly population that dies of pneumonia each year (Brunnström, 2009). As dementia progresses, parts of the brain responsible for proper swallowing and breathing are damaged making it more likely for patients to cough or choke. Patients also become weaker as the disease progresses; this may cause the muscles required for proper swallowing to malfunction. Individuals with swallowing problems are more likely to aspirate saliva, food, and drinks which can lead to fatal aspiration pneumonia (Payne, 2018).

With dementia causing a growing burden on the number of individuals living with the disease, on their loved ones, and on the global economy and healthcare systems, there remains a question of what causes dementia and if there are any possible treatments to help those currently affected by the disorder (NIH Neurological Disorders and Stroke). Because of this question, the U.S. government alone invests over $3.1 billion annually to research the mechanisms and potential treatments of dementia (Alzheimer's Association, 2020). A growing field within dementia research

is focused on understanding the connection between oral health and the disorder (Lee, 2019; Zenthöfer, 2014; Noble, 2013; Stewart, 2015).

Oral health and dementia

Can oral health really influence brain health? Recent studies suggest that oral health and cognitive health may have a bi-directional relationship. Cognitive decline could lead to poor oral health and poor oral health may precede poor cognitive health. Patients with dementia may be unable to effectively perform oral hygiene tasks, due to dexterity complications, or forget altogether to include them in their daily routine. This can lead to quick oral health decline (Pazos, 2016; Ghezzi, 2000). Meanwhile, epidemic studies suggest that individuals with gum disease and mouth infections are more likely to develop dementia later in life (Nadim, 2020). Studies have also shown that patients with dementia have on average fewer natural teeth, more cavities, tooth decay, plaque, and periodontal disease compared to cognitively healthy individuals (Lee, 2019; Zhang, 2020; Ellefsen, 2007; Ide, 2016).

Tooth loss is being suggested as a risk factor for decreased cognitive function in elderly patients (Fang, 2018; Lee, 2019). According to a 2021 meta-analysis by Qi et al., of 14 studies, totaling 34,074 participants and 4,689 cases, subjects with tooth loss had 1.48 times more risk of developing cognitive impairment. The analysis also found a dose-dependent risk with each tooth lost equating to 1.48% increased risk of developing cognitive decline and 1.11% increased risk of being diagnosed with dementia. Additionally, the analysis found that complete loss of teeth (edentulism) results in a 1.54 times increased risk of cognitive impairment and a 1.40 times increased risk of being diagnosed with dementia (Qi, 2021). Another study by Zhang et al. evaluated 102 individuals and

determined that an increasing number of missing teeth was associated with a worse Mini-Mental State Exam (MMSE) score, indicating greater cognitive decline. They found that individuals with seven or more missing teeth had significantly worse cognitive ability than those missing six or less teeth (Zhang, 2020), other studies report similar findings (Kamer, 2012; Stein, 2007; Grabe 2009; Okamoto, 2017; Ranjan, 2019). Although these studies are mostly correlational, mechanisms are being proposed and studied to better understand if this relationship is causal and how it may work. Chia-Shu Lin has proposed that tooth loss may lead to decreased sensory feedback to the brain, leading to cognitive decline. Sensory information while chewing, stimulates areas of the brain like the hippocampus (a key area in memory formation), so when teeth are missing, less stimulation results in decreased hippocampal activity and neuronal degeneration. Another hypothesis by Weijenberg et al. proposes that impaired chewing ability may lead to nutrient deficiencies important for proper nervous system function. Particularly, a decrease in B-vitamins, which are often deficient in dementia patients (Morris, 2012). More research is needed to determine if tooth loss has a causal relationship with dementia, and if so, what the mechanism of this relationship is.

Tooth decay is also found to be more prevalent in patients with dementia. A 1993 cross-sectional study by Jones *et al.*, found that patients with dementia had double the number of coronal caries and over seven times the number of root caries compared to cognitively healthy controls. Although the study evaluates a small number of patients, multiple other studies with larger sample sizes have since reproduced these results (Chalmers, 2002; Delwel, 2017; Chen, 2013). Tooth decay, also called dental caries or cavities, refers to damage to the mineralization of teeth that can affect both the root and coronal area of a tooth (Machiulskiene, 2020; Saunders, 2005). Cavities are caused by decay-causing bacteria residing on the teeth. These bacteria consume carbohydrates and sugars

ingested by the person and as a result produce acids that break down tooth enamel (outer layer of the tooth). If untreated, decay progresses to the inner layers, eventually reaching the innermost layer, the pulp, which is connected to the bloodstream (Rathee, 2021; Li, 2017). Once in the bloodstream, bacteria can travel to other organs and increase systemic inflammation (Hajishengallis, 2021).

Bacteria is also responsible for gum disease, better known as periodontal disease, the most prevalent disease of the oral cavity in the general adult and elderly populations, and with an even higher prevalence in patients with dementia. (Nazir, 2017; Gil-Montoya, 2015; Rai, 2010; Martande, 2014). A 2018 meta-analysis by Gusman et al. showed a significant association between periodontal disease and dementia, though a question remains about the direction of the association (Leira, 2017). A 2021 cohort study by Ma et al. of 8,640 patients with dementia and 8,640 matched controls showed that patients with dementia were significantly more likely to develop periodontal disease over a ten-year period than patients without dementia. In addition, a growing body of evidence supports the idea that periodontal disease precedes dementia (Farhad, 2014; Stein, 2007). A 2020 longitudinal cohort study of 8,275 subjects by Demmer et al. found that individuals with severe periodontal disease in adulthood had about 20% greater incidence of dementia at a 20-year follow-up compared to those without periodontal disease. Periodontal disease is characterized by inflammation of the gums, which causes the gums to detach from the teeth and recede creating pockets. The periodontal membrane and alveolar bone are damaged as periodontal disease progresses, and often the disease results in tooth loss (Coventry, 2000). Periodontal disease often begins when bacterial biofilms called plaque and tartar form and sit on the surface of the teeth (Nazir, 2017; Albandar, 2000). Many bacterial species have been identified to be responsible for periodontal disease and they include: *Porphyromonas gingivalis, Tannerella forsynthia, Treponema denticola, Filifactor alocis,* and

Actinomyces actinomycetemcomitans, as well as bacteria from the Synergistetes and Peptostreptococcaceae taxonomic groups (Socransky, 1998; Holt, 2005; Griffen, 2012; Abusleme, 2013; Slots, 1980; Haubek, 2008). Plaque forming bacteria sit on the teeth, infect the gums, and cause its inflammation and destruction, changing the local environment to allow even more pathogenic bacteria to grow (Socransky, 2000; Sedghi, 2021). It is hypothesized that the epithelial cells of the gums react to the bacteria present by releasing immune factors to recruit immune cells from systemic circulation. The proteolytic response from the recruited immune cells then causes damage to the epithelium allowing the bacteria to invade deeper, reach the bloodstream, and increase systemic inflammation (Bosshardt, 2005).

There is a strong correlation between caries, periodontal disease, and tooth loss and cognitive decline. All three factors are influenced by pathogenic bacteria present in the oral cavity, and severe forms of these oral health problems may be associated with increased systemic inflammation. For this reason, there is a growing interest in understanding how the oral microbiome may influence systemic inflammation and brain health and how it could explain the oral health-dementia bi-directional association.

Microbiome

The phrase "You are not alone" has never been truer. Most people think about themselves as unique, independent and, many times, lonely, individuals, without realizing that they live with 100 trillion microbes. These bacteria (the most studied), fungi, viruses, and archaea live in complex communities across the human body (Amon, 2017), the compositions of which are influenced by genetics and environmental factors (Schroeder, 2016). Which of these microbes (and where and

when they colonize) are beneficial or harmful to our health is still unknown. To study the microbiome, researchers divide bacterial groups based on their body location. The microbiome can be found in multiple places across the "outside" of the human body such as on the skin, in the nose and oral cavity, in the digestive tract (gut), and in the vagina.

In health, signals from these microbes modulate important functions of the human body such as host immunity through lipopolysaccharides (LPS), host dietary fiber degradation, gut motility, appetite through short-chain fatty acids (SCFAs), and host metabolism through bile acids (BAs) (Schroeder, 2016). The microbiome also plays a role in proper brain development and function and plays an active role in governing several aspects of Central Nervous System (CNS) like the physiology, glial cell maturation, and behavior (Abdel-Haq, 2019). Studies done in germ-free mice have shown the microbiome is involved in maturation and diversity of microglia (immune macrophage cells in the CNS) proper neuron myelination, and normal stress response (Erny, 2017; Hoban, 2016; Luczynski, 2016). The microbiome regulates these aspects of the CNS through physical and chemical connections within blood vessels and nerve pathways (Mayer, 2015). Research suggests that neuroactive metabolites from bacteria consisting of GABA, tryptophan precursors, catecholamines, and serotonin can interact directly with receptors on adjacent host cells or travel via the bloodstream or the vagal and spinal nerves to receptors in the brain (Mayer, 2015).

When pathogenic bacteria grow in abundance and commensal, or protective, bacteria decrease in abundance the microbiome becomes dysbiotic and disease may result (Schippa, 2014). For example, the inflammatory cytokines released into the bloodstream in response to pathogenic bacteria have been shown to negatively affect insulin producing cells in Type I diabetes and increase

insulin resistance in Type II diabetes. (Gulden, 2016; Monro, 2016). A dysbiotic microbiome can lead to liver disease, as the bile acids produced by the liver are not processed properly by opportunistic, pathogenic bacteria which leads to liver inflammation (Milosevic, 2019). Bacteria have also been implicated in Inflammatory Bowel Disease (IBD). Studies have shown permeability increase in the intestines, commonly seen in IBD, may be a result of bacteria causing apoptosis to the intestinal barrier cells (Benjamin, 2008). With a seemingly large role in proper brain development and overall systemic health, as well as a large role in inflammatory disorders, it is plausible that the microbiome may also be involved in inflammatory disorders of the brain, such as in dementia.

Microbiome and dementia

A compelling hypothesis for the initiation and progression of neurodegenerative disorders, such as AD, proposes that systemic inflammation may affect neuroinflammation and promote neurodegeneration. A large, representative cohort study of 623 surviving cases of sepsis, an extreme reaction of the body to an infection that damages the body's own tissues, found that sepsis is independently linked to cognitive impairment and those who survive severe sepsis have tripled odds of developing moderate/severe dementia. This study suggests further that about 20,000 new cases of moderate/severe cases of cognitive impairment may be result of sepsis (Iwashyna, 2012). There are key characteristics of neuroinflammation in response to infection like activated immune cells and alteration of cytokine and chemokine levels, all of which are seen in dementia patients. Activated microglia are important in the immune response to neurodegeneration. Studies by Cagnin et al. suggest that activated macrophage response is an early event in the pathogenesis of dementia (Cagnin, 2001). A 2021 study by Asby et al. found that systemic infection contributes to dementia

by altering brain cytokine levels and exacerbating cerebral hypoperfusion and blood–brain barrier leakiness which are both associated with multiple diseases of dementia (Asby, 2021). Essentially, dementia patients present with immune responses in the brain and neuroinflammation consistent with infection which points to bacteria as being a culprit in the mechanisms resulting in dementia.

Most of these studies showing a connection between the microbiome and the brain have been studied in what is commonly called the ”gut-brain axis.” The gut brain axis is a bi-directional relationship through which the bacteria of the gastrointestinal (GI) tract and the CNS influence one another and communicate (Kawalski, 2019). Bacteria in the gut can influence the CNS via nerve pathways, immune signaling, endocrine signaling, and metabolic signaling (Quigley, 2017). Research suggests that dysbiosis of the gut may affect this communication and influence neuroinflammation that leads to dementia. As we age and in disease, it has been shown the gut is more likely to have a greater abundance of harmful bacteria (Kawalski, 2019). These pathogenic bacteria of the gut have been shown to increase systemic inflammation which leads to neuroinflammation and neuronal death (Perry, 2013; Cantteneo, 2017).

Oral bacteria and dementia

The gateway to the gut is the mouth, could oral health and oral bacteria also affect neuroinflammation? It would be wrong for research to focus solely on the gut and ignore the oral microbiome's possible role in dementia onset and progression, as the oral microbiome often influences the composition of the gut microbiome, mostly when the oral microbiome is dysbiotic. Oral bacteria enter the stomach by way of swallowing food, drinks, and saliva. Once in the stomach, most oral bacteria are unable to survive under the harsh acidic conditions, athough, many pathogenic oral bacteria are more acid-resistant and can thrive in the stomach, such as *Porphyromonas gingivalis* (Kato, 2018). A study done in mice by Nakajima in 2015 showed that oral administration of pathogenic oral bacteria resulted in gut dysbiosis and increased endotoxin (a toxin present in bacterial cells) levels in blood which preceded further systemic inflammation in the mice.

Recent research has provided further support for a connection between the oral microbiome and dementia, of which the mechanisms and association are not yet known. Both LPS (Poole, 2013) and DNA (Dominy, 2019) of *P. gingivalis* have been found in the autopsy specimen of brains of patients with AD. In addition, post-mortem studies by Dominy also found gingipains, toxic proteases from oral bacteria, in the brains of patients with AD. These gingipains were found in greatest quantities in the areas of the brain involved in memory, like the hippocampus, and their quantity correlated with severe forms of AD pathologies. This same study also found the DNA of *P. gingivalis* in the cerebral spinal fluid (CSF) of patients with AD (Dominy, 2019). These studies suggest that *P. gingivalis* and oral bacteria metabolites could cross the blood brain barrier (BBB)

into the brains of patients with AD, potentially affecting neuroinflammation; although the mechanism and whether this infiltration occurs before or after the onset of AD is still unknown.

Much of the evidence suggesting a connection between the oral microbiome and dementia comes from correlational studies. The first major study finding evidence of a causational link between the oral microbiome and dementia was published by Dominy in 2019. Oral infection of mice with *P. gingivalis* resulted in colonization of the bacteria in the brains of the mice, as well as resulted in increased production of amyloid-beta $(A\beta)_{1–42}$, an important component of Aβ plaques of the AD pathology, neuroinflammation, active immune cells, tau tangles, and neurodegeneration. This study also suggests the involvement of *P. gingivalis* and its gingipains as involved in the tau pathology of AD, as they show gingipains directly damage tau through proteolysis and by activating human proteases that then also affect tau. Oral introduction of gingipain inhibitors in-vivo "reduced the bacterial load of an established *P. gingivalis* brain infection, blocked $A\beta_{1–42}$ production, reduced neuroinflammation, and rescued neurons in the hippocampus" (Dominy, 2019). This study gives promising evidence of a possible treatment for AD.

A limitation to the previous research study is that it focuses on only one pathogenic oral bacteria species. It is important for additional research to continue to determine what other bacteria may be involved in the pathogenesis of dementia. Current research on the oral microbiome composition in patients with dementia has found high abundance of opportunistic, pathogenic bacteria in the mouths of patients with dementia to include bacteria of the genera and phyla *Lactobacillus*, *Streptococcus*, *Firmicutes*, *Bacteroidetes*, *Leptotrichia*, and *Fusobacteriaceae* (Wu, 2021; Cockburn, 2012; Bathini, 2020). Limitations to these studies include their small sample size,

cross-sectional design, and the fact these studies did not also look at oral health factors that may be affecting the bacterial composition in the oral cavity.

Current research is also exploring how bacteria can cross anatomical barriers and invade the brain. It is hypothesized that bacteria may cross the epithelial cell barrier in the oral cavity and then travel to the brain through the blood vessels or along the cranial nerves, such as the olfactory nerve (Talamo, 1991). Another hypothesis suggests that pathogenic bacteria travel down the digestive tract and then climb up the vagus nerve to the brain and/or enter the bloodstream through the intestinal epithelial cell barrier. Once inside blood vessels or on a nerve, bacteria can travel to the brain by damaging the blood brain barrier. Sheets et al. showed that bacterial proteases cause damage and even death to endothelial cell adhesion, making cell barriers leakier and easier to bypass (Sheets, 2005). Another proposed mechanism, the "Trojan horse," suggests that bacteria may access the brain indirectly once in the bloodstream by infecting immune cells like monocytes, which can cross the blood brain barrier (Cavrois, 2008).

Research has shown increasing evidence that oral bacteria can research the brain in several ways, increasing neuroinflammation, and possibly leading to dementia onset and progression.

Summary of thesis

Dementia is an incurable and devastating disease affecting millions worldwide. The difficulty we face to understand the onset and progression of this disease may be due to the complexity of factors involved. It is becoming increasingly clear that chronic or prolonged systemic inflammatory problems may be a key factor in understanding and preventing this disorder.

Therefore, systematic documentation of the human microbiome in health and disease is necessary. The objective of this study is to understand the current oral health status of individuals with various levels of dementia living in the community and nursing homes. The following study will further explore the relationship of dementia, oral health, and oral bacteria. We will analyze oral health and oral bacteria composition across varying levels of cognitive ability and across residency type. Our findings show that there is worse oral health as dementia progresses and worse oral health in patients with dementia residing in nursing homes. Focusing on improving the oral health in patients with dementia in the community and in nursing homes can help to prevent further oral and systemic diseases common to those living with dementia.

When comparing across cognitive level, it would be ideal to compare a range of cognitive levels within the community or nursing home. For example, it would be ideal to compare those in the community with no cognitive impairment to those with mild dementia and then to severe dementia. This could also be done in the nursing home setting. Though not ideal, in this study we lack the community dwelling (CD) Severe group due to a smaller proportion of dementia patients overall being in the stage and living in the community, and nursing home (NH) controls groups, due to all participants in the nursing home having some level of cognitive decline, needed to make these ideal comparisons.

When comparing across residency type it would be ideal to compare individuals with no cognitive decline in both the community and in nursing homes, individuals with mild dementia in both the community and nursing home, and individuals with severe dementia in both the community and nursing home. Again due to limitations in sample population discussed in the previous

paragraph, this study was only able to compare individuals with mild dementia across residency type.

The main hypothesis is that level of dementia and residence type will impact the oral health of patients with dementia. To study this hypothesis, we created two specific aims:

Specific Aim 1. Determine how oral health changes with level of cognitive status.

Hypothesis: *The oral health of community dwelling participants will decline with decreasing cognitive ability.* **Approach:** We will perform a standardized oral health assessment of community dwelling patients with dementia who visit the UTHealth Neurocognitive Disorders Clinic. We will compare this data across levels of cognitive impairment and to the data of healthy controls. Further, we will also collect data on the individuals' oral hygiene habits, oral health, perceived oral health, swallowing ability, and systemic health as these can have a relationship with overall oral health. In addition, we will use next-generation sequencing to determine the participants' oral microbiome to see if cognitive status impacts bacteria composition.

Impact: Aim 1 will provide a baseline understanding of the relationship between oral health and varying levels of cognitive status, to include cognitively healthy individuals.

Specific Aim 2: Determine how oral health changes with residency type.

Hypothesis: *The oral health of nursing home residents with dementia will be worse compared to community dwelling patients with dementia.* **Approach:** Utilizing the same approaches as aim 1, we will determine the oral health of nursing home residents with dementia from two Houston-area nursing homes. We will compare the oral health and oral microbiome data to that of

the community dwelling patients with dementia to determine if one residency type has greater influence on oral health and oral bacteria composition.

Impact: Aim 2 will provide evidence for which residency type best promotes the oral health of patients with dementia and will inform better oral hygiene protocols and adherence to slow the progression of oral health decline in either residency type.

Overall Impact: This study aims to determine differences in oral health among patients with differing levels of dementia and place of residence. Previous research suggests a connection between oral health and dementia status. This is the first study to determine if place of residence has an impact on the oral health of patients with dementia. This work is important because it will provide information on multiple factors impacting the oral health of patients with dementia and lead to research identifying specific mechanisms between oral health decline and dementia.

Few studies have analyzed oral health and oral microbiome in relationship with dementia status. The significance of understanding these associations has been best described by Lire-Junior *et al*. in the 2018 commentary article "Oral-gut connection: one step closer to an integrated view of the gastrointestinal tract?". The author states "One remarkable contribution to the field would be to identify the oral health status of the included individuals in the study… In fact, this has frequently been overlooked in studies assessing the relationship between oral microbiota and systemic diseases" (Junior, 2018).

CHAPTER 2: METHODS AND MATERIALS

Study subjects and design

A total of 52 subjects participated in this cross-sectional case-control study. From a random sampling of community dwelling individuals, 22 participants met all the criteria and participated in the data collection. The subjects were recruited from the Neurocognitive Disorders Center (NDC) of the McGovern Medical School at UTHealth. For each subject, we performed a standardized oral screening examination (**Appendix A – Kayser Jones Brief Oral Health Status Examination (BOSHE)**), plaque index (**Appendix B – Simplified Oral Hygiene Index (OHI-S)**), neurocognitive assessment (**Appendix C – Montreal Cognitive Assessment (MOCA)**), and completed questionnaires regarding oral hygiene (**Appendix D – Oral Hygiene Habits Questionnaire**), dietary habits (**Appendix E – Dietary Habits Questionnaire**), and swallowing ability (**Appendix F – RADBOUD Oral Motor Inventory**). At this visit we also collected each subject's medical history. All participants from the NDC were recruited between June 2019 and December 2021. From a random sampling of nursing home residents, 30 participants met all the criteria and participated in data collection. The subjects were recruited from two Houston-area assisted living facilities, namely, The Towers at Bayou Manor and Colonial Oaks Memory Care at Braeswood. We performed the same oral screening examination, plaque index, and neurocognitive assessment. The nursing home residents completed the same questionnaires and medical history as the community dwelling participants. All participants from the nursing home were recruited between April 2019 and December 2019.

Inclusion and exclusion criteria

Inclusion criteria included (1) age 50 years and older and (2) no complaints of cognitive decline (control) or Montreal Cognitive Assessment (MOCA) score < 26.

Exclusion criteria included (1) radiation therapy to the head or neck, (2) smoking history, (3) antibiotic use in the three months prior to sample collection.

Demographic and oral health data collection

After participants consented to the study, general demographic information was collected including age, sex, race, ethnicity, primary language, and education level. We collected participants' medical history and neurological history from updated medical charts if available (If not available, patients provided this information during their visit).

All participants completed four standardized questionnaires (oral hygiene habits, dietary habits, saliva, and swallowing questionnaire of the RADBOUD Oral Motor Inventory (ROMP)), and underwent an oral health screening comprised of the Brief Oral Health Status Examination (BOHSE) and the Simplified Oral Hygiene Index (OHI-S). Salivary pH was measured with pH paper indicator dipped in an unstimulated saliva sample.

Oral bacteria sample collection

At least one hour after brushing or eating, two soft tissue sites and two hard tissue sites (listed below) were sampled using Catch-All^{TM} Sample Collection Swabs (Epicentre Biotechnologies, Madison, WI). One swab was used for the soft tissue sample and one for the hard tissue sample. If a

patient did not have some of the teeth (e.g., missing, dentures) designated for the hard tissue sample, the hard tissue swab was only to be taken of those hard tissue sites that were present.

Immediately after sampling, the swabs were swirled in a Mo Bio Power Bead tube (small 2 mL screw-top tube containing 750 µL specimen collection fluid [50mM Tris buffer pH 7.6, 1mM EDTA pH 8, and 0.5% Tween-20]). The swab sponge was pressed against the tube wall and floor (into the red garnet beads) multiple times for 20 seconds to ensure transfer of bacteria from the swab to the solution. The samples were then stored at -80° C until sent to the Baylor College of Medicine Alkek Center for Metagenomics and Microbiome Research for 16s rRNA analysis.

Collection sites and instructions

Soft Tissue (use only one swab for all areas)

1. Tongue dorsum: Swab 1 cm^2 of the center of the tongue for 5 seconds.
2. Buccal mucosa: Swab the entire area of both left and right buccal mucosa for 5 seconds each. Take care not to touch the teeth.

Hard Tissue (use only one swab for all areas)

1. Lingual side of lower incisors: Swab the plaque on the lingual side of the lower incisors for 5 seconds.
2. Top of molars: Swab the top of all molars for 5 seconds.

CHAPTER 3: DOES ORAL HEALTH CHANGE BY LEVEL OF COGNITIVE DECLINE?

Demographics and medical history

We recruited eleven patients attending the UTHealth Neurocognitive Disorders Center diagnosed with mild cognitive impairment or mild dementia according to the Montreal Cognitive Assessment. We grouped these patients together to create the CD Mild group. We also recruited eleven patient caregivers and family members with no complaints of cognitive impairment as control subjects to create the CD Control group. Table 1 shows demographic information of both groups. There were no significant differences in age (Tukey's HSD, p-value = 1) or gender (chi-square test of independence, χ^2 (1, N = 22) = 0.18, p = 0.670), and no difference in race and ethnicity (p-value = 1) between community dwelling patients with dementia and controls (**Table 1**). There were no significant differences in prevalence of diabetes (chi-square test of independence, χ^2 (1, N = 21) = 2.432, p = 0.119) or hypertension (chi-square test of independence, χ^2 (1, N = 21) = 0.064, p = 0.800) between community dwelling patients with dementia and controls. A history of heart disease was more prevalent in community dwelling patients with dementia (chi-square test of independence, χ^2 (1, N = 21) = 9.545, p = 0.0002) (**Table 1)**.

Table 1: Demographic and comorbidity information of community dwelling subjects

	CD Control (n = 11)	**CD Mild (n = 11)**	**p-value**[†]
Age (Years, mean ± SD)	69.2 ± 5.6	69.2 ± 8.9	1
Gender N (%)			0.670
Male	6 (55)	5 (45)	
Female	5 (45)	6 (55)	
Race/Ethnicity N (%)			1
White	11 (100)	11 (100)	
Diabetes N (%)	2 (20) [‡]	0 (0)	0.119
Hypertension N (%)	4 (40) [‡]	5 (45)	0.800

Heart Disease N (%)	0 (0) ‡	7 (64)	0.0002*

CD: Community dwelling. † Chi-square calculated for categorical data and Tukey's Honestly Significant Difference for continuous data, ‡ One participant's data unknown, * $p < 0.05$.

We recruited 30 nursing home residents of which 12 were diagnosed with mild cognitive impairment or mild dementia. We grouped these residents together to create the NH Mild group. Eighteen residents were diagnosed with moderate or severe dementia and grouped to create the NH Severe group. Table 2 shows demographic and medical information of both groups. There were no significant differences in age (Tukey's HSD, p-value = 0.732), gender (chi-square test of independence, χ^2 (1, N = 30) = 0.455, p = 0.500), or race and ethnicity (chi-square test of independence, χ^2 (2, N = 30) = 1.556, p = 0.459) between nursing home residents with mild and severe dementia (**Table 2**). There were no differences in prevalence of diabetes (chi-square test of independence, χ^2 (1, N = 30) = 1.000, p = 0.317), hypertension (chi-square test of independence, χ^2 (1, N = 30) = 0.238, p = 0.626), or heart disease (chi-square test of independence, χ^2 (1, N = 30) = 1.094, p = 0.296) between nursing home residents with mild and severe dementia (**Table 2**).

Table 2: Demographic and comorbidity information of nursing home participants

	NH Mild (n = 12)	**NH Severe (n = 18)**	**p-value†**
Age (Years, mean ± SD)	88.5 ± 5.6	85.5 ± 7.8	0.300
Gender N (%)			0.500
Male	4 (33)	4 (22)	
Female	8 (67)	14 (88)	
Race/Ethnicity N (%)			0.459
White	11 (92)	14 (78)	
Asian	1 (8)	2 (11)	
Black	0 (0)	2 (11)	
Diabetes N (%)	3 (25)	2 (11)	0.317
Hypertension N (%)	9 (75)	12 (67)	0.626
Heart Disease N (%)	7 (58)	7 (39)	0.296

NH: Nursing home. † Chi-square calculated for categorical data and Tukey's Honestly Significant Difference for continuous data.

Measures of oral health across cognitive status

i. Oral screening

When community dwelling participants with mild dementia (CD mild) were compared to controls (CD controls) there were no differences in oral health measured by the BOSHE oral health screening (Tukey's HSD, p = 0.989) (**Figure 1A**). When comparing between nursing home residents with mild dementia (NH Mild) and severe dementia (NH Severe) there were no differences in oral health measured by the BOSHE oral health screening (Tukey's HSD, p = 0.098) (**Figure 1B**).

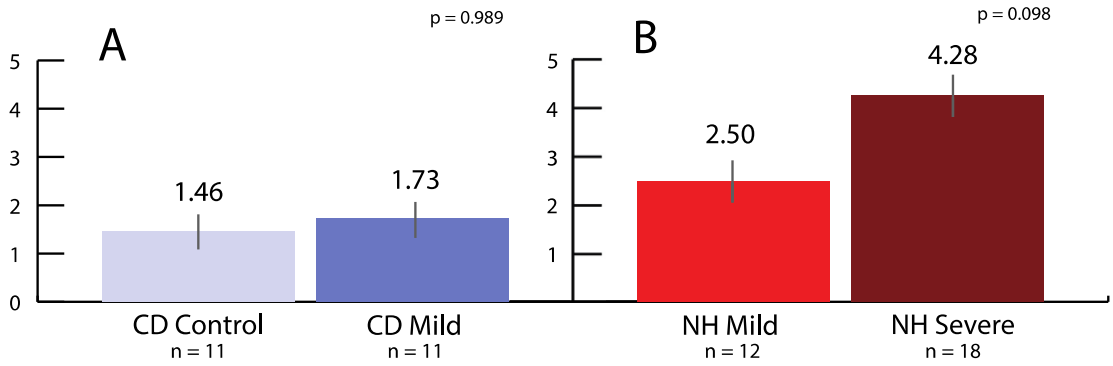


Figure 1: Oral screening scores across cognitive status. (**A**) Bar graph shows no difference in oral health according to the BOSHE oral screening between community dwelling participants with no cognitive impairment and mild dementia (Tukey's HSD, p = 0.989). (**B**) Bar graph shows no difference in oral health according to the BOSHE oral screening between nursing home residents with mild and severe dementia (Tukey's HSD, p = 0.098). Greater score indicates worse oral health.

As a part of the oral health screening, we also counted the number of natural teeth of each participant. We found no difference in number of natural teeth across cognitive ability. When community dwelling participants with mild dementia were compared to community dwelling participants with no cognitive impairment there was no difference in number of natural teeth (chi-square test of independence, χ^2 (3, N = 20) = 3.704, p = 0.295). When nursing home residents with

mild dementia were compared to nursing home residents with severe dementia, we found no difference in number of natural teeth (chi-square test of independence, χ^2 (3, N = 30) = 2.179, p = 0.536) (**Table 3**).

Table 3: Number of teeth across cognitive status

	CD Control (n = 09)	**CD Mild (n = 11)**	**p-value†**
Severity of tooth loss: N (%)			0.295
No tooth loss (28-32 teeth)	4 (44)	2 (18)	
Mild tooth loss (24-27 teeth)	2 (22)	6 (55)	
Moderate tooth loss (17-23)	3 (33)	2 (18)	
Severe tooth loss (1-16)	0 (0)	1 (09)	
Edentulous (0 teeth)	0 (0)	0 (0)	

	NH Mild (n = 12)	**NH Severe (n = 18)**	**p-value†**
Severity of tooth loss: N (%)			0.536
No tooth loss (28-32 teeth)	5 (42)	8 (44)	
Mild tooth loss (24-27 teeth)	4 (33)	6 (33)	
Moderate tooth loss (17-23)	3 (25)	2 (11)	
Severe tooth loss (1-16)	0 (0)	2 (11)	
Edentulous (0 teeth)	0 (0)	0 (0)	

CD: Community dwelling, NH: Nursing home. † Chi-square calculated for categorical data.

Since a tooth is less effective during mastication, or chewing, when it is not paired with a tooth on the opposite jaw, we counted the number of chewing pairs present in each participant's oral cavity. This count provides more information than the number of teeth in how effectively participants are able to chew. Across cognitive ability we found no differences in the number of chewing pairs of teeth. When community dwelling patients with mild dementia were compared to community dwelling subjects with no cognitive impairment, there was no difference in number of chewing pairs (chi-square test of independence, χ^2 (1, N = 22) = 2.200, p = 0.138). When nursing

home residents with mild dementia were compared to nursing home residents with severe dementia, there was no difference in number of chewing pairs (chi-square test of independence, χ^2 (2, N = 30) = 1.624, p = 0.444) (**Table 4**).

Table 4: Abundance of chewing pairs across cognitive status

	CD Control (n = 11)	**CD Mild (n = 11)**	**p-value**[†]
Quantity of chewing pairs: N (%)			0.138
≥ 12 chewing pairs	9 (82)	11 (100)	
8-12 chewing pairs	2 (18)	0 (0)	
≤ 7 chewing pairs	0 (0)	0 (0)	

	NH Mild (n = 12)	**NH Severe (n = 18)**	**p-value**[†]
Quantity of chewing pairs: N (%)			0.444
≥ 12 chewing pairs	7 (58)	8 (44)	
8-12 chewing pairs	5 (42)	8 (44)	
≤ 7 chewing pairs	0 (0)	2 (12)	

CD: Community dwelling, NH: Nursing home. [†] Chi-square calculated for categorical data.

ii. Plaque index

When community dwelling subjects with mild dementia were compared to controls, there were no differences in oral health measured by the OHI-S plaque index (Tukey's HSD, p = 0.991) (**Figure 2A)**. When nursing home residents with mild and severe dementia were compared, there were no differences in oral health measured by the OHI-S plaque index (Tukey's, p-Value = 0.2) (**Figure 2B**).

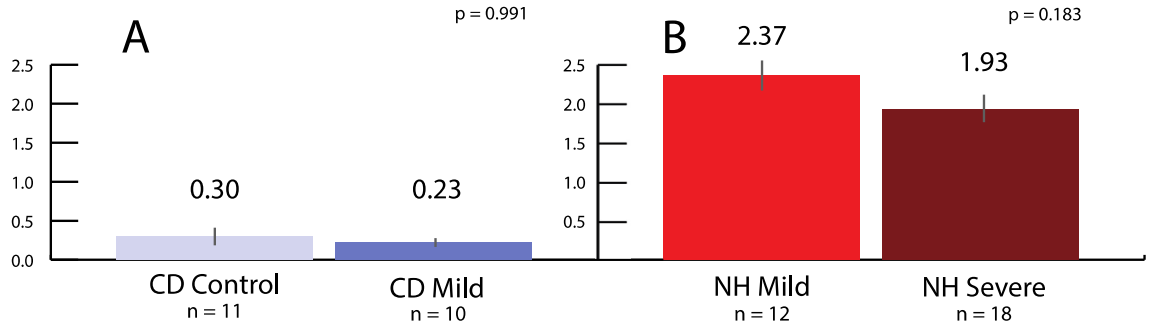


Figure 2: OHI-S plaque index across cognitive status. (**A**) Bar graph shows no difference in oral health according to the OHI-S plaque index between community dwelling participants with no cognitive impairment and mild dementia (Tukey's HSD, p = 0.991). (**B**) Bar graph shows no difference in oral health according to the OHI-S plaque index between nursing home residents with mild and severe dementia (Tukey's, p-Value = 0.2). Greater score indicates more plaque on the teeth.

iii. Oral bacteria abundance and composition

Hard tissue samples were analyzed and compared between individuals in the community with mild dementia (CD Mild) and individuals with no cognitive impairment (CD Control). Alpha diversity did not significantly differ between the hard tissue samples of the groups (Kruskal-Wallis rank sum, p-Value = 1), indicating a similar number of species are found on the hard tissue across these groups (**Figure 3**).

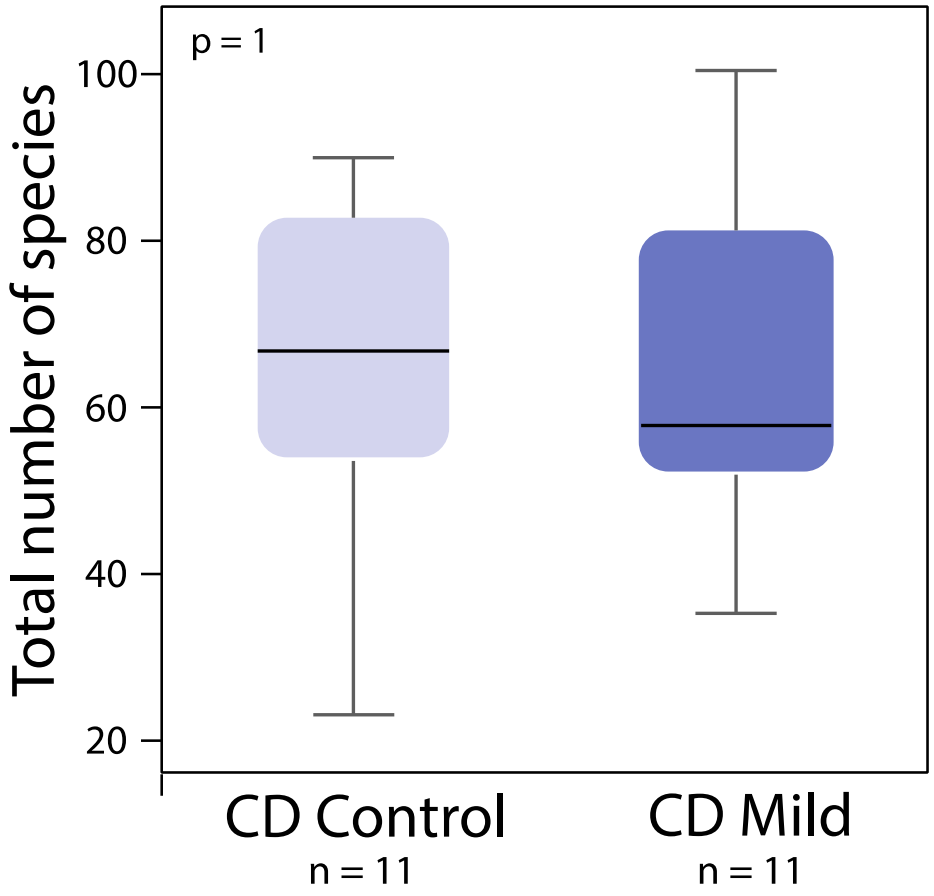


Figure 3: Alpha Diversity of hard tissue samples from community dwelling subjects with no cognitive impairment and mild dementia. Alpha diversity was compared between hard tissue samples of community dwelling patients with mild dementia and community dwelling individuals

with no cognitive impairment. Graph shows no difference in alpha diversity between groups (Kruskal-Wallis rank sum, p-Value = 1).

Soft tissue samples were analyzed and compared between community dwelling individuals with mild dementia and no cognitive impairment. Alpha diversity did not significantly differ between the soft tissue samples of the groups (Kruskal-Wallis rank sum, p-Value = 0.7), indicating a similar number of species are found on the soft tissue across these groups (**Figure 4**).

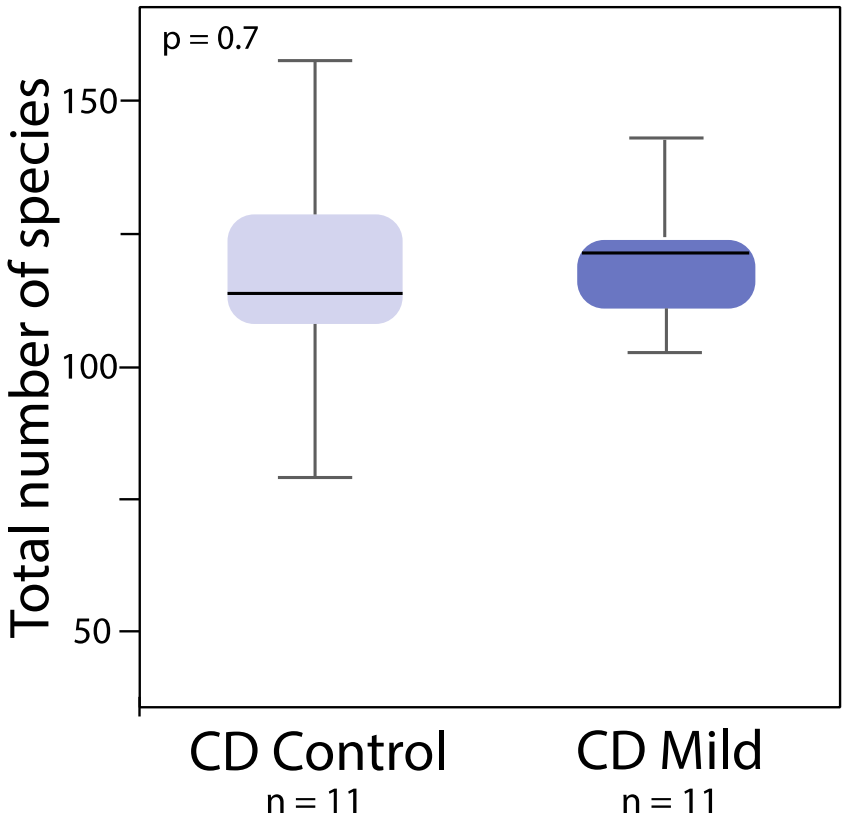


Figure 4: Alpha diversity of soft tissue samples from community dwelling subjects with no cognitive impairment and mild dementia. Alpha diversity was compared between soft tissue samples from community dwelling subjects with mild and no dementia. Graph shows no difference in alpha diversity between groups (Kruskal-Wallis rank sum, p-Value = 0.7).

We next measured beta diversity differences using PERMANOVA. We measured beta diversity differences by comparing relative species abundances between samples (Bray Curtis distance), species presence and absence between samples (Jaccard distance), relative abundance of phylogenetic lineages (Weighted Unifrac), and presence/absence of phylogenetic lineages (Unweighted Unifrac). We found the beta diversity did not differ between CD Mild and CD Control groups' hard tissue samples according to Bray Curtis distance (PERMANOVA, $p = 0.429$), Jaccard distance (PERMANOVA, $p=0.437$), Weighted Unifrac (PERMANOVA, $p= 0.967$), and Unweighted Unifrac (PERMANOVA, $p=0.162$) (**Figure 5A-D**).

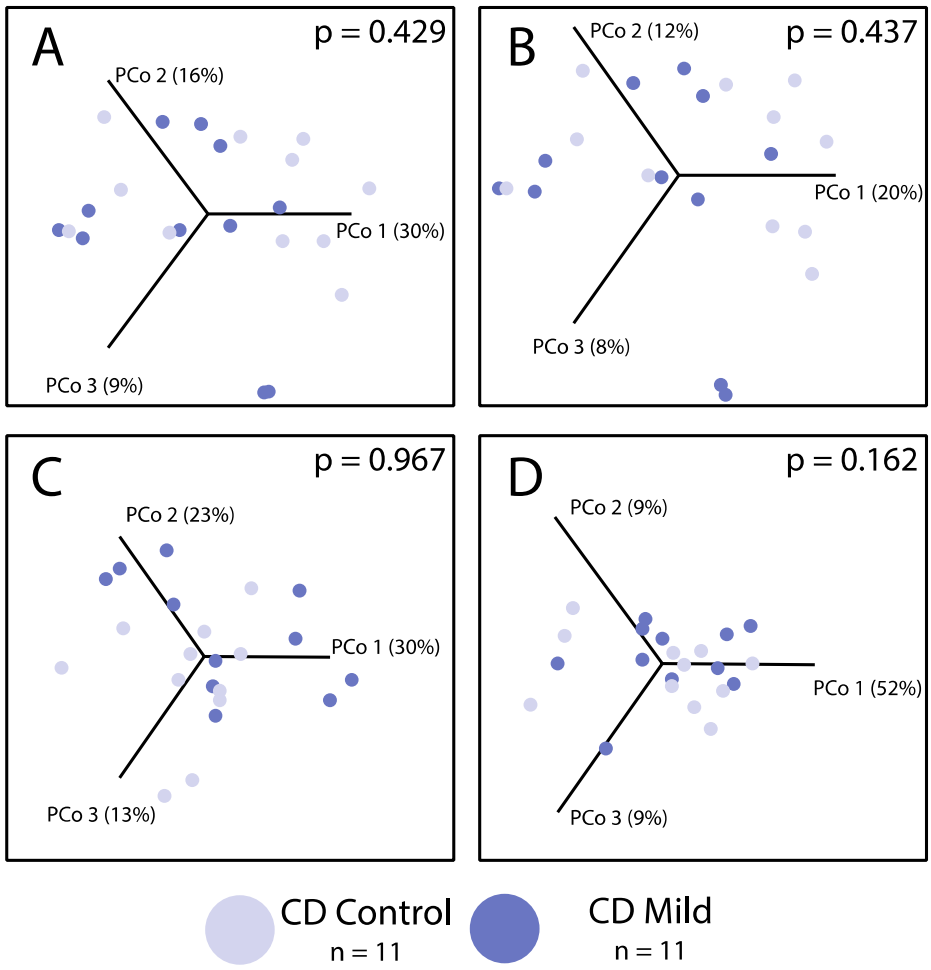


Figure 5: Beta diversity of the hard tissue samples between community dwelling subjects with no cognitive impairment and mild dementia represented on Principal Component (Pco) plots. Samples from CD Control and CD Mild subjects show no significant differences in bacterial communities according to (A) Bray Curtis distance (PERMANOVA, p = 0.429), (B) Jaccard distance (PERMANOVA, p=0.437), (C) Weighted Unifrac (PERMANOVA, p= 0.967), and (D) Unweighted Unifrac (PERMANOVA, p=0.162).

We next measured beta diversity differences of the soft tissue samples between the CD Control and CD Mild subjects. We found the beta diversity did not differ between CD Mild and CD Control groups' soft tissue samples according to Bray Curtis distance (PERMANOVA, p = 0.310),

Jaccard distance (PERMANOVA, p=0.369), Weighted Unifrac (PERMANOVA, p= 0.841), and Unweighted Unifrac (PERMANOVA, p=0.265) (**Figure 6A-D**).

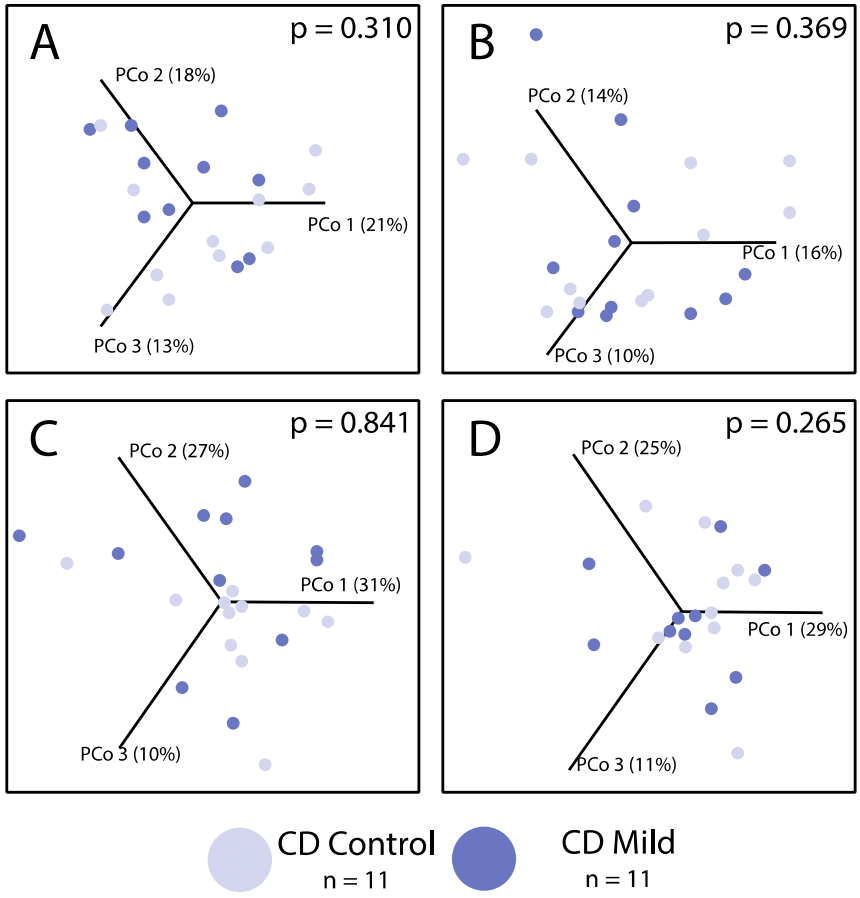


Figure 6: Beta diversity of the soft tissue samples between community dwelling subjects with no cognitive impairment and mild dementia represented on PCo plots. Samples from CD Control and CD Mild subjects show no significant differences in bacterial communities of the soft tissues according to (**A**) Bray Curtis distance (PERMANOVA, p = 0.310), (**B**) Jaccard distance (PERMANOVA, p=0.369), (**C**) Weighted Unifrac (PERMANOVA, p= 0.841), and (**D**) Unweighted Unifrac (PERMANOVA, p=0.265).

Hard tissue samples were analyzed and compared between nursing home residents with mild dementia (NH Mild) and severe dementia (NH Severe). Alpha diversity did not significantly differ between the hard tissue samples of the groups (Kruskal-Wallis rank sum, p-Value = 0.2), indicating a similar number of species are found on the hard tissue across these groups (**Figure 7**).

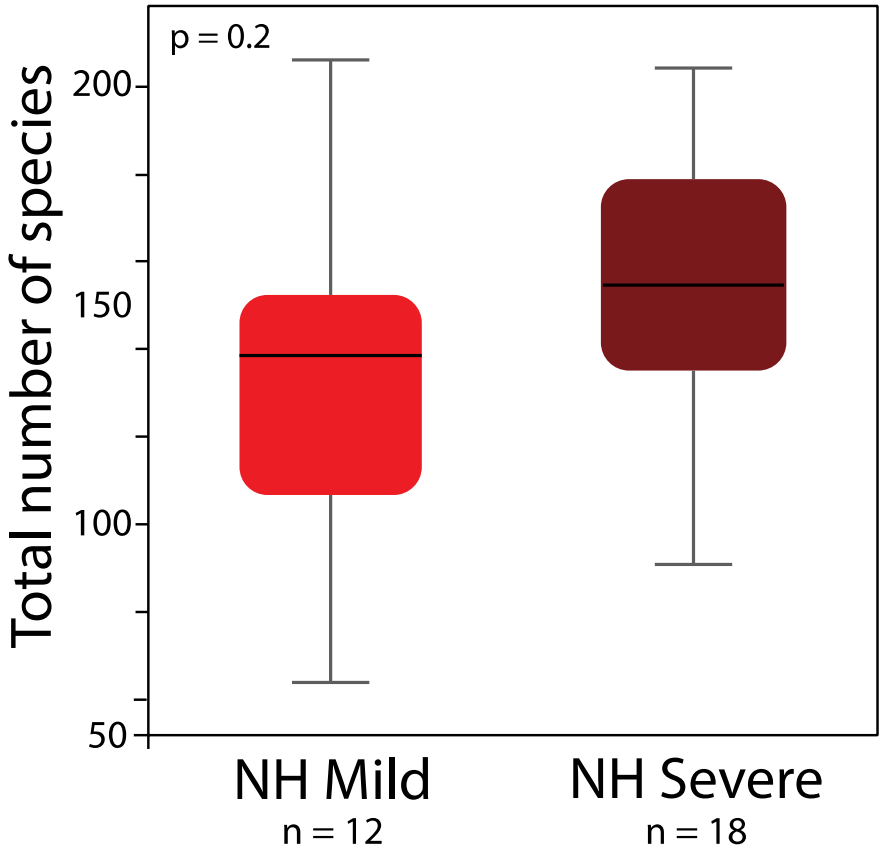


Figure 7: Alpha diversity of hard tissue samples of nursing home residents with mild and severe dementia. Graph shows no difference in alpha diversity between groups (Kruskal-Wallis rank sum, p-Value = 0.2).

Soft tissue samples were analyzed and compared between nursing home residents with mild dementia and severe dementia. Alpha diversity did not significantly differ between the soft tissue

samples of the groups (Kruskal-Wallis rank sum, p-Value = 0.2), indicating a similar number of species are found on the soft tissue across these groups (**Figure 8**).

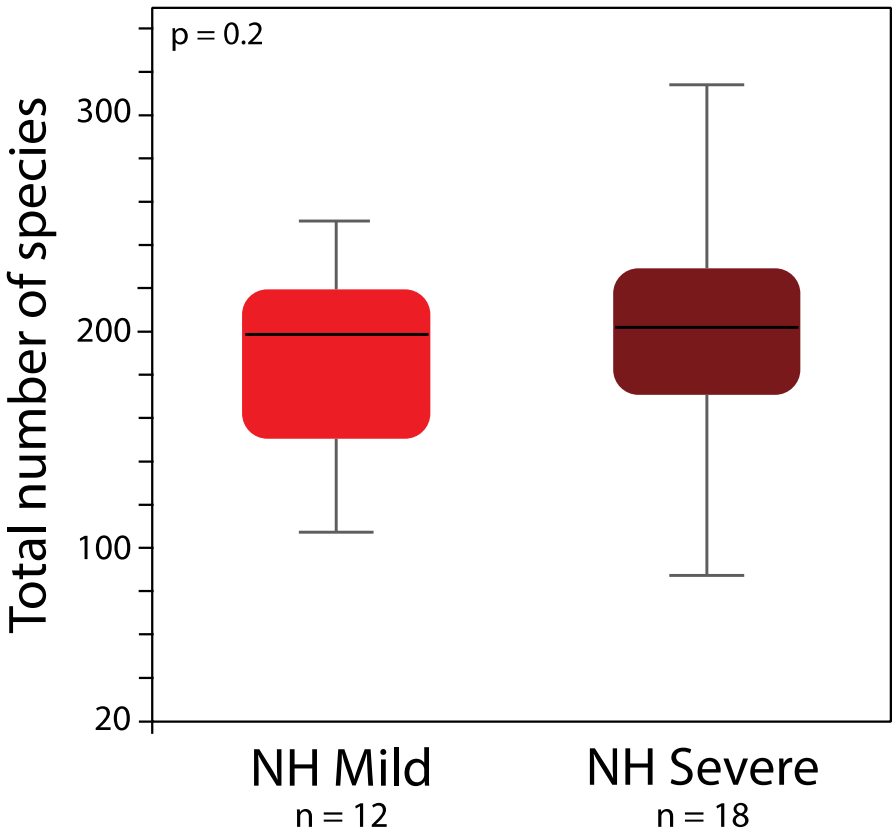


Figure 8: Alpha diversity of soft tissue samples from nursing home residents with mild and severe dementia. Graph shows no difference in alpha diversity between groups (Kruskal-Wallis rank sum, p-Value = 0.2).

Comparing between NH Mild and NH Severe groups' hard tissue samples we found the beta diversity differed according to Bray Curtis distance (PERMANOVA, p = 0.012), Jaccard distance (PERMANOVA, p=0.012), and Weighted Unifrac (PERMANOVA, p= 0.009). Beta diversity did not differ between NH Mild and NH Severe groups' hard tissues samples according to Unweighted Unifrac (PERMANOVA, p=0.1) (**Figure 9A-D**).

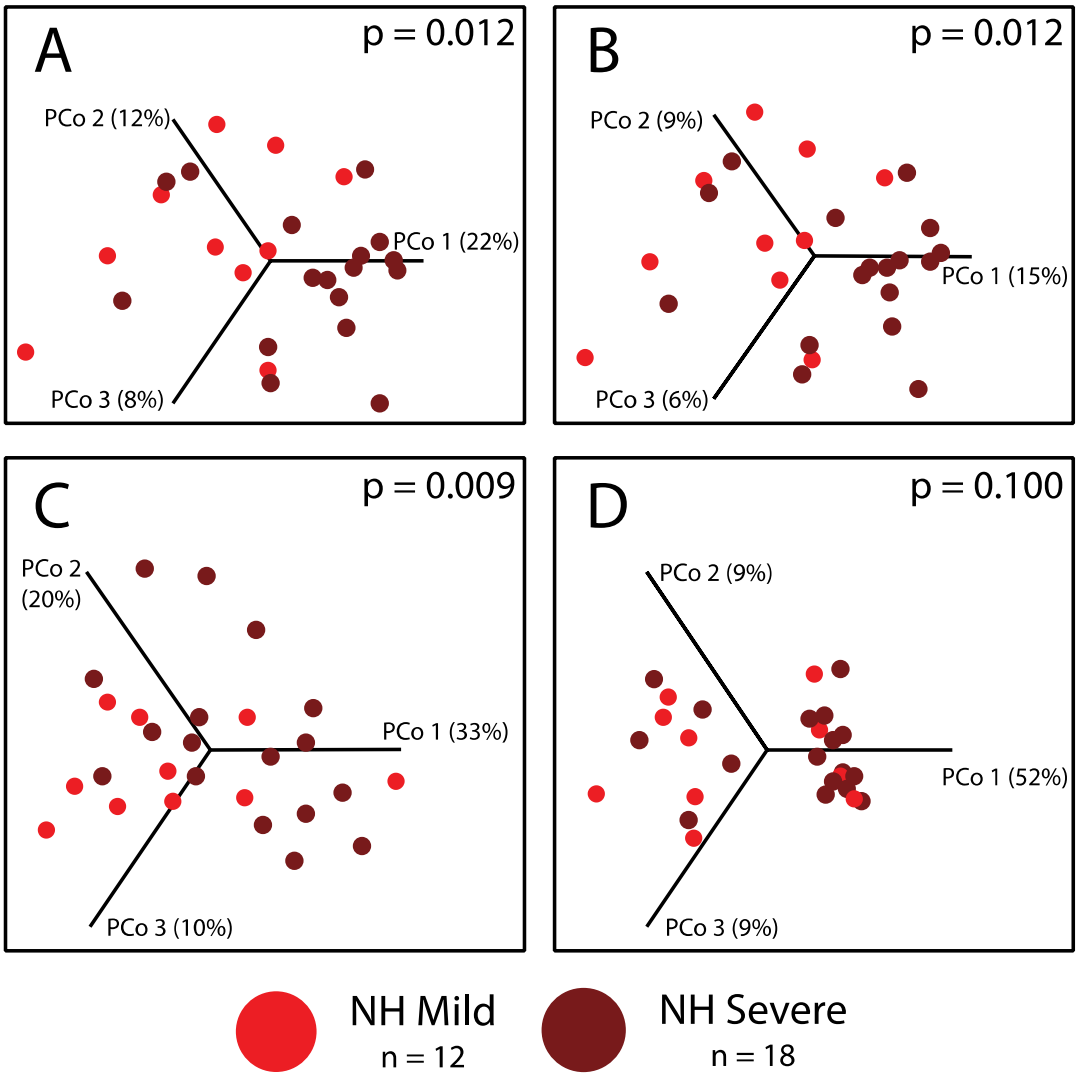


Figure 9: Beta diversity of the hard tissue samples between nursing home residents with mild and severe dementia represented on PCo plots. Samples from NH Mild and NH Severe subjects show significantly different bacterial communities according to (**A**) Bray Curtis distance (PERMANOVA, p = 0.012), (**B**) Jaccard distance (PERMANOVA, p=0.012), and (**C**) Weighted Unifrac (PERMANOVA, p= 0.009). Hard tissue samples of the NH Mild and NH Severe subjects show no difference in bacterial communities according to (**D**) Unweighted Unifrac (PERMANOVA, p=0.1).

We found that 98 species significantly differed between the NH Mild and NH Severe groups' hard tissue samples (**Figure 10**). Hard tissue samples from NH Severe participants had greater abundance of pathogenic bacteria of the genera *Provetella, Lactobacillus, and Treponema.*

Figure 10: Relative abundance of bacteria species that significantly differ on hard tissues between nursing home residents with mild and severe dementia. The abundance of 98 bacteria

species significantly differs on the hard tissues between nursing home residents with mild and severe dementia.

Assessing for differences in bacteria composition between NH Mild and NH Severe groups' soft tissue samples, we found the beta diversity differed according to Unweighted Unifrac (PERMANOVA, p=0.03). The beta diversity did not differ between NH Mild and NH Severe groups' soft tissue samples according to Bray Curtis distance (PERMANOVA, p = 0.72), Jaccard distance (PERMANOVA, p=0.66), and Weighted Unifrac (PERMANOVA, p= 0.27) (**Figure 11A-D**).

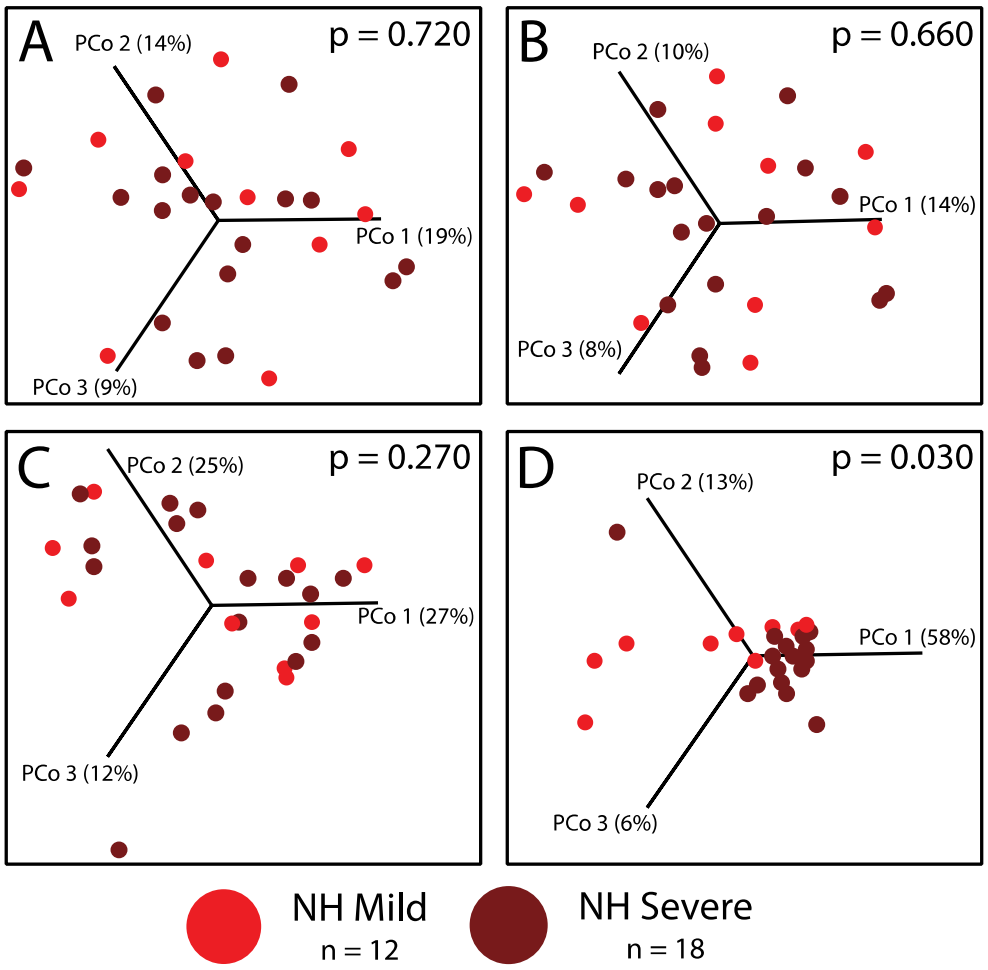


Figure 11: Beta diversity of the soft tissue samples between nursing home residents with mild and severe dementia represented on PCo plots. Samples from NH Mild and NH Severe subjects show no significant differences of bacterial communities according to (**A**) Bray Curtis distance (PERMANOVA, p = 0.72), (**B**) Jaccard distance (PERMANOVA, p=0.66), and (**C**) Weighted Unifrac (PERMANOVA, p= 0.27). Soft tissue samples from NH Mild and NH Severe subjects show significant differences in bacterial communities according to (**D**) Unweighted Unifrac (PERMANOVA, p=0.03).

We found an abundance of 29 species significantly differed between the NH Mild and NH Severe groups' soft tissue samples (**Figure 12**). Of the different species, NH Severe samples had greater abundance of pathogenic bacteria *Lactobacillus salivarius*, *Streptococcus downei*, and *Streptococcus pneumoniae.*

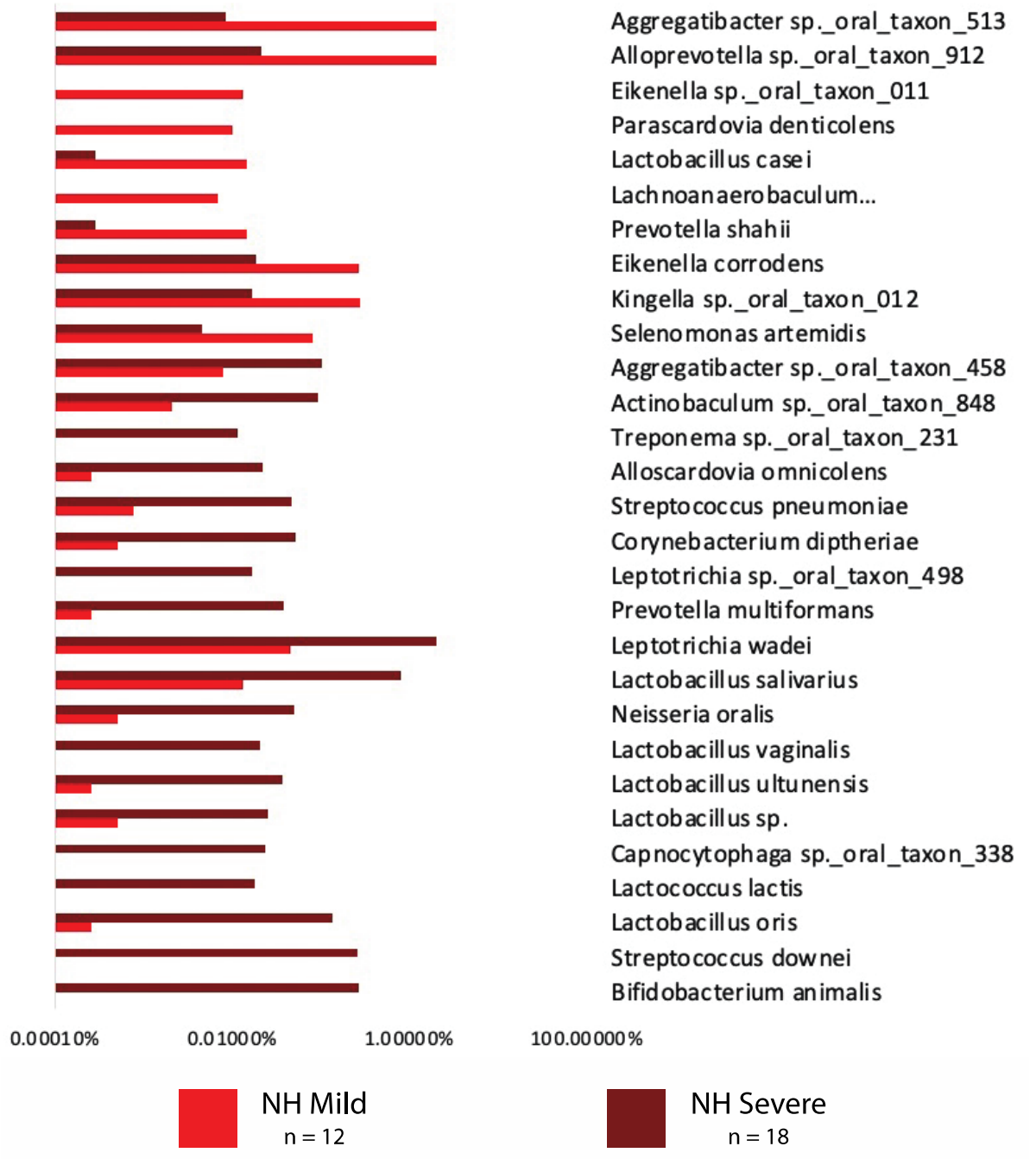


Figure 12: Relative abundance of bacteria that significantly differ on the soft tissues between nursing home residents with mild and severe dementia. The abundance of 29 bacteria species significantly differs on the soft tissues between nursing home residents with mild and severe dementia.

Oral health factors affecting oral health across cognitive status

i. Oral hygiene habits

We utilized an oral hygiene habits questionnaire to measure general oral hygiene habits across cognitive ability. Across cognitive level, there were no differences in general oral hygiene. Between the CD Control and CD Mild groups there were no differences in toothbrushing frequency (chi-square test of independence, χ^2 (1, N = 22) = 0.259, p = 0.611), flossing frequency (chi-square test of independence, χ^2 (1, N = 22) = 0.000, p = 1), and dental visit frequency (chi-square test of independence, χ^2 (1, N = 22) = 2.651, p = 0.266). Comparing across nursing home residents with mild and severe dementia there were no differences in toothbrushing frequency (chi-square test of independence, χ^2 (2, N = 30) = 0.455, p = 0.797), flossing frequency (chi-square test of independence, χ^2 (2, N = 30) = 2.380, p = 0.304), and dental visit frequency (chi-square test of independence, χ^2 (2, N = 30) = 0.680, p = 0.712) (**Table 5**).

Table 5: Oral hygiene habits across cognitive status

	CD Control (n = 11)	**CD Mild (n = 11)**	**p-value**[†]
Tooth brushing N (%)			0.611
Meets or exceeds daily recommendation (≥2x/day)	8 (73)	9 (82)	
Below recommendation	3 (27)	2 (18)	
Never	0 (0)	0 (0)	
Flossing N (%)			1
Meets or exceeds daily recommendation (≥1X/day)	6 (55)	6 (55)	
Below recommendation	4 (36)	4 (36)	
Never	1 (09)	1 (09)	
Dental visit frequency N (%)			0.266
Meets or exceeds yearly recommendation (≥2x/year)	6 (55)	8 (73)	
Below recommendation	2 (18)	3 (27)	
Never	3 (27)	0 (0)	

	NH Mild (n = 12)	NH Severe (n = 18)	p-value†
Tooth brushing N (%)			0.797
Meets or exceeds daily recommendation (≥2x/day)	8 (67)	14 (78)	
Below recommendation	3 (25)	3 (17)	
Never	1 (08)	1 (05)	
Flossing N (%)			0.304
Meets or exceeds daily recommendation (≥1X/day)	4 (33)	7 (39)	
Below recommendation	3 (25)	1 (05)	
Never	5 (42)	10 (56)	
Dental visit frequency N (%)			0.712
Meets or exceeds yearly recommendation (≥2x/year)	3 (25)	5 (28)	
Below recommendation	7 (58)	8 (44)	
Never	2 (17)	5 (28)	

CD: Community dwelling, NH: Nursing home. † Chi-square calculated for categorical data.

ii. Assistance when performing oral hygiene tasks

We asked all participants if they required any assistance while performing oral hygiene tasks. From both the CD Control and CD Mild groups, there were no study subjects that required any assistance while performing oral hygiene tasks (p-value = 1). The NH Severe group required significantly more assistance while performing oral hygiene tasks compared to the NH Mild group (chi-square test of independence, χ^2 (1, N = 30) = 4.537, p = 0.033) (**Table 6**).

Table 6: Requirement of assistance for oral hygiene across cognitive status

	CD Control (n = 11)	CD Mild (n = 11)	p-value†
Require assistance with oral hygiene tasks N (%)	0 (0)	0 (0)	1

	NH Mild (n = 12)	NH Severe (n = 18)	p-value†
Require assistance with oral hygiene tasks N (%)	2 (17)	10 (56)	0.033*

CD: Community dwelling, NH: Nursing home. † Chi-square calculated for categorical data. * $p < 0.05$.

iii. Oral cleanliness

During the oral screening, we also observed the oral cleanliness of all participants by noting how many places there were food particles and tartar. There were no differences in oral cleanliness based on food particles and tartar density in the oral cavity between community dwelling participants with mild dementia and controls (chi-square test of independence, χ^2 (2, N = 22) = 5.333, p = 0.069) or between nursing home residents with mild and severe dementia (chi-square test of independence, χ^2 (2, N = 30) = 2.540**, p = 0.281) (Table 7).**

Table 7: Oral cleanliness across cognitive status

	CD Control (n = 11)	CD Mild (n = 11)	p-value†
Food particles/tartar in: N (%)			0.069
No places	10 (91)	5 (45)	
1-2 places	1 (09)	5 (45)	
≥3 places	0 (0)	1 (10)	

	NH Mild (n = 12)	NH Severe (n = 18)	p-value†
Food particles/tartar in: N (%)			0.281
No places	6 (50)	4 (22)	
1-2 places	2 (17)	4 (22)	
≥3 places	4 (33)	10 (56)	

CD: Community dwelling, NH: Nursing home. † Chi-square calculated for categorical data.

iv. Dietary habits

I utilized a dietary habits questionnaire to measure general dietary habits across cognitive ability. Comparing community dwelling participants with mild dementia to controls we found no difference in a requirement of a modified soft food diet (chi-square test of independence, χ^2 (1, N = 22) = 1.048, p = 0.306), number of meals (chi-square test of independence, χ^2 (1, N = 22) = 0.733, p = 0.392), snacks (chi-square test of independence, χ^2 (2, N = 22) = 0.277, p = 0.871), sweets (chi-square test of independence, : χ^2 (1, N = 22) = 0.917, p = 0.338), carbohydrates (chi-square test of independence, χ^2 (2, N = 22) = 1.222, p = 0.543), and sugary drinks (chi-square test of independence, χ^2 (2, N = 22) = 1.491, p = 0.475) consumed per day (**Table 8**).

Comparing between nursing home residents with mild and severe dementia, we found significantly more residents with severe dementia require a modified soft food diet (chi-square test of independence, χ^2 (2, N = 30) = 7.273, p = 0.026). Between the residents with mild and severe dementia, we found no differences in daily consumption of meals (chi-square test of independence, χ^2 (1, N = 30) = 1.552, p = 0.213), snacks (chi-square test of independence, χ^2 (2, N = 30) = 0.825, p = 0.622), sweets (chi-square test of independence, χ^2 (2, N = 30) = 1.667, p = 0.435), carbohydrates (chi-square test of independence, χ^2 (1, N = 30) = 0.370, p = 0.543) or sugary drinks (chi-square test of independence, χ^2 (2, N = 30) = 2.055, p = 0.358) (**Table 8**).

Table 8: Dietary habits across cognitive status

	CD Control (n = 11)	**CD Mild (n = 11)**	**p-value†**
Requires a modified, soft food diet N (%)	1 (09)	0 (0)	0.306
Meals per day N (%)			0.392
<3 meals/day	6 (55)	4 (36)	
3 meals/day	5 (45)	7 (64)	
>3 meals/day	0 (0)	0 (0)	
Snacks per day N (%)			0.871

≤1 snack/day	6 (55)	7 (64)	
2 snacks/day	3 (27)	2 (18)	
≥3 snacks/day	2 (18)	2 (18)	
Sweet foods per day N (%)			0.338
No sweets/day	2 (18)	4 (36)	
1-2 sweets/day	9 (82)	7 (64)	
≥3 sweets/day	0 (0)	0 (0)	
Carbs per day N (%)			0.543
No carbs/day	1 (09)	2 (18)	
1-2 carb/day	9 (82)	6 (55)	
≥3 carbs/day	1 (09)	3 (27)	
Sugary drinks per day N (%)			0.475
No drinks/day	5 (45)	6 (55)	
1-2 drink/day	6 (55)	4 (36)	
≥3 drinks/day	0 (0)	1 (09)	

	NH Mild (n = 12)	**NH Severe (n = 18)**	**p-value†**
Requires a modified, soft food diet N (%)	0 (0)	8 (44)	0.026*
Meals per day N (%)			0.213
<3 meals/day	1 (8)	0 (0)	
3 meals/day	11 (92)	18 (100)	
>3 meals/day	0 (0)	0 (0)	
Snacks per day N (%)			0.622
≤1 snack/day	8 (67)	9 (50)	
2 snacks/day	3 (25)	7 (39)	
≥3 snacks/day	1 (08)	2 (11)	
Sweet foods per day N (%)			0.435
No sweets/day	3 (25)	6 (33)	
1-2 sweets/day	8 (67)	12 (67)	
≥3 sweets/day	1 (08)	0 (0)	
Carbs per day N (%)			0.543
No carbs/day	4 (33)	8 (44)	
1-2 carb/day	8 (67)	10 (56)	
≥3 carbs/day	0 (0)	0 (0)	
Sugary drinks per day N (%)			0.358
No drinks/day	3 (33)	9 (50)	
1-2 drink/day	8 (59)	9 (50)	
≥3 drinks/day	1 (8)	0 (0)	

CD: Community dwelling, NH: Nursing home. † Chi-square calculated for categorical data. * $p < 0.05$.

v. Salivary pH

During the oral health screening, we measured the salivary pH of all subjects. We found no difference in salivary pH across cognitive decline. There were no differences in salivary pH between community dwelling participants with mild dementia and controls (Student's T-test, $p = 0.638$) (**Figure 13A**) or between nursing home residents with mild and severe dementia (Student's T-test, $p = 0.379$) (**Figure 13B**). The mean salivary pH for the community dwelling controls, community dwelling patients with mild dementia, and nursing home residents with mild dementia was within the normal salivary pH range of 6.2-7.6. The mean salivary pH for the NH Severe group is slightly more acidic than the normal salivary pH range.

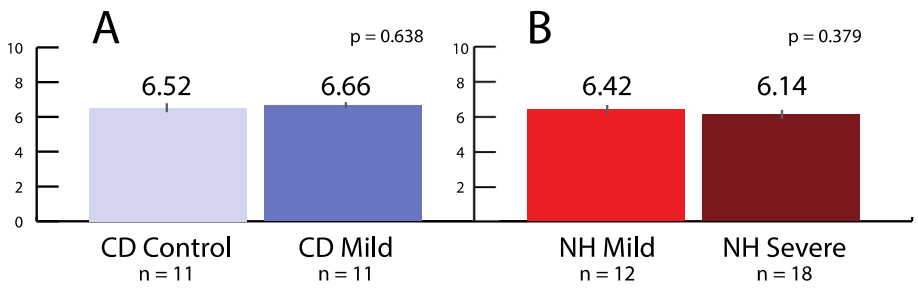


Figure 13: Salivary pH across cognitive status. (**A**) Bar graph shows no difference of salivary pH between community dwelling participants with no cognitive impairment and mild dementia (Student's T-test, $p = 0.638$). (**B**) Bar graph shows no difference of salivary pH between nursing home residents with mild and severe dementia.

vi. Swallowing ability

I utilized the RADBOUD oral motor inventory questionnaire to determine if subjects have any difficulty while swallowing. Comparing community dwelling patients with dementia to controls, we found there was no difference in swallowing ability (chi-square test of independence, χ^2 (1, N = 22) = 1.048, p = 0.306). When comparing nursing home residents with mild and severe dementia, we found that the residents with severe dementia had significantly worse swallowing ability compared to residents with mild dementia (chi-square test of independence, χ^2 (2, N = 30) = 7.056, p = 0.029). Residents with severe dementia did not choke overall more often (chi-square test of independence, χ^2 (1, N = 30) = 1, p = 0.317) or have more trouble drinking (chi-square test of independence, χ^2 (2, N = 30) = 4.431, p = 0.109) compared to residents with mild dementia, but they did report having more trouble swallowing while eating (chi-square test of independence, χ^2 (2, N = 30) = 7.130, p = 0.028) and taking pills (chi-square test of independence, χ^2 (2, N = 30) = 10.000, p = 0.007) (**Table 9**).

Table 9: RADBOUD swallowing ability questionnaire across cognitive status

	CD Control (n = 11)	**CD Mild (n = 11)**	**p-value†**
Swallowing difficulty N (%)			0.306
None	11 (100)	11 (91)	
Mild	0 (0)	1 (9)	
Severe	0 (0)	0 (0)	

	NH Mild (n = 12)	**NH Severe (n = 18)**	**p-value†**
Swallowing difficulty N (%)			0.029*
None	11 (92)	8 (44)	
Mild	1 (8)	7 (39)	
Severe	0	3 (17)	
Choking frequency (%)			0.317
Not regularly	11 (92)	14 (78)	
1X/week	1 (8)	3 (17)	

1X/day	0 (0)	1 (5)	
Swallowing difficulty while drinking (%)	12 (100)	12 (67)	0.109
None	0 (0)	5 (28)	
Mild	0 (0)	1 (5)	
Severe			
Swallowing difficulty while eating (%)			0.028
None	11 (92)	8 (45)	
Mild	1 (8)	6 (33)	
Severe	0 (0)	4 (22)	
Swallowing difficulty with taking pills (%)	12 (100)	8 (44)	0.007
None	0 (0)	8 (44)	
Mild	0 (0)	2 (12)	
Severe			

CD: Community dwelling, NH: Nursing home. † Chi-square calculated for categorical data. * $p < 0.05$.

CHAPTER 4: DOES ORAL HEALTH STATUS CHANGE WITH RESIDENCY TYPE?

Demographics and medical history

To compare oral health across residency type, we are utilizing the eleven patients recruited from the UTHealth Neurocognitive Disorders Center who were diagnosed with mild cognitive impairment or mild dementia (CD Mild) and the twelve nursing home residents recruited diagnosed with mild cognitive impairment or mild dementia (NH Mild). Table 10 shows demographic information of both groups. Comparing across residency type, the nursing home residents were significantly older than the community dwelling patients (Tukey's HSD, $p<0.0001$). There were no significant differences in gender (chi-square test of independence, χ^2 (1, N = 23) = 0.354, p = 0.552) or race and ethnicity (chi-square test of independence, χ^2 (1, N = 23) = 0.958, p = 0.328) between community dwelling patients with mild dementia and nursing home residents with mild dementia (**Table 10**). There were no differences in prevalence of diabetes (chi-square test of independence, χ^2 (1, N = 23) = 3.163, p = 0.328), hypertension (chi-square test of independence, χ^2 (1, N = 23) = 0.068, p = 0.075), or heart disease (chi-square test of independence, χ^2 (1, N = 23) = 2.103, p = 0.147) between community dwelling patients with mild dementia and nursing home residents with mild dementia (**Table 10**).

Table 10: Demographic and comorbidity information of participants with mild dementia

	CD Mild (n = 11)	**NH Mild (n = 12)**	**p-value**[†]
Age (Years, mean ± SD)	69.2 ± 8.9	88.5 ± 5.6	<0.0001[*]
Gender N (%)			.552
Male	5 (45)	4 (33)	
Female	6 (55)	8 (67)	
Race/Ethnicity N (%)			.328
White	11 (100)	11 (92)	
Asian	0 (0)	1 (8)	

Diabetes N (%)	0 (0)	3 (25)	.075
Hypertension N (%)	5 (45)	9 (75)	.147
Heart Disease N (%)	7 (64)	7 (58)	.795

CD: Community dwelling, NH: Nursing home. † Chi-square calculated for categorical data and Tukey's Honestly Significant Difference for continuous data. * $p < 0.05$.

Measures of oral health

i. Oral screening

Comparing community dwelling patients and nursing home residents with mild dementia there were no differences in oral health measured by BOSHE across residency type (Tukey's HSD, p = 0.769) (**Figure 14**).

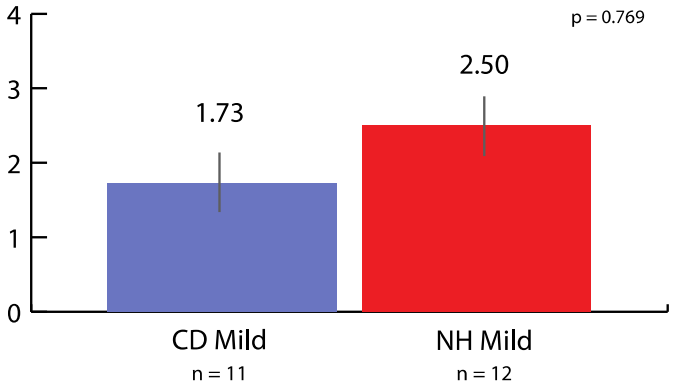


Figure 14: Oral screening score across residency type. Bar graph shows no difference in oral health according to the BOSHE oral screening between community dwelling patients and nursing home residents with mild dementia (Tukey's HSD, p = 0.769). Greater score indicates worse oral health.

When comparing community dwelling subjects with mild dementia to nursing home residents with mild dementia, we found no difference in the number of natural teeth present in the oral cavity (chi-square test of independence, χ^2 (3, N = 21) = 2.848, p = 0.416) (**Table 11**).

Table 11: Number of teeth across residency type

	CD Mild (n = 11)	**NH Mild (n = 12)**	**p-value**[†]
Severity of tooth loss: N (%)			0.416
No tooth loss (28-32 teeth)	2 (18)	5 (42)	
Mild tooth loss (24-27 teeth)	6 (55)	4 (33)	

Moderate tooth loss (17-23)	2 (18)	3 (25)	
Severe tooth loss (1-16)	1 (09)	0 (0)	
Edentulous (0 teeth)	0 (0)	0 (0)	
	CD Mild (n = 11)	**NH Mild (n = 12)**	**p-value**[†]
Severity of tooth loss: N (%)			0.416
No tooth loss (28-32 teeth)	2 (18)	5 (42)	
Mild tooth loss (24-27 teeth)	6 (55)	4 (33)	
Moderate tooth loss (17-23)	2 (18)	3 (25)	
Severe tooth loss (1-16)	1 (09)	0 (0)	
Edentulous (0 teeth)	0 (0)	0 (0)	

CD: Community dwelling, NH: Nursing home. [†] Chi-square calculated for categorical data.

A difference in number of chewing pairs was found between community dwelling and nursing home patients with mild dementia (chi-square test of independence, χ^2 (1, N = 23) = 5.856, p = 0.016). The community dwelling subjects all had the ideal twelve or more chewing pairs present. Significantly more nursing home residents with mild dementia had fewer than twelve chewing pairs (**Table 12**).

Table 12: Abundance of chewing pairs across residency type

	CD Mild (n = 11)	**NH Mild (n = 12)**	**p-value**[†]
Quantity of chewing pairs: N (%)			0.016[*]
≥ 12 chewing pairs	11 (100)	7 (58)	
8-12 chewing pairs	0 (0)	5 (42)	
≤ 7 chewing pairs	0 (0)	0 (0)	

CD: Community dwelling, NH: Nursing home. [†] Chi-square calculated for categorical data. [*] $p < 0.05$.

ii. Plaque index

As measured by OHI-S plaque index, nursing home residents had greater accumulation of tooth plaque than the community dwelling participants (Tukey's HSD, $p < 0.0001$) (**Figure 15**).

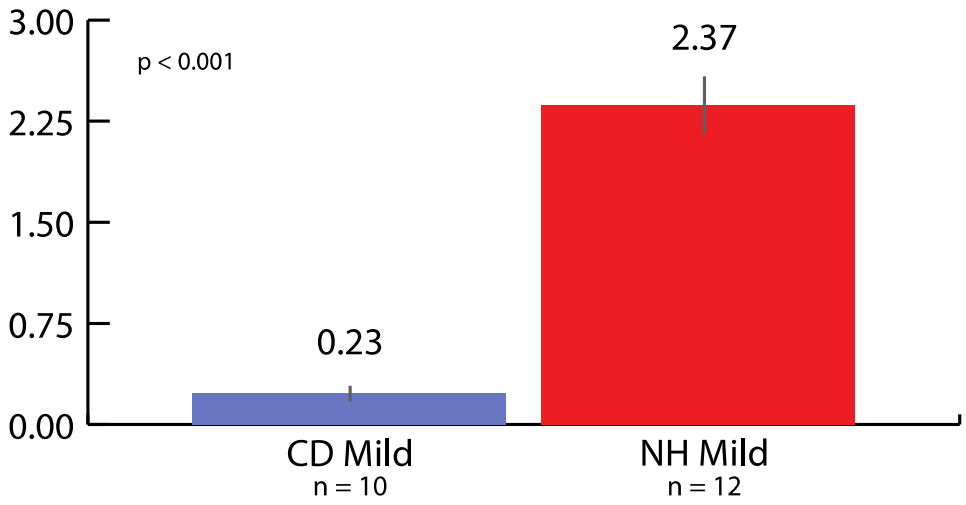


Figure 15: Plaque index across residency type. Bar graph shows a difference in oral health according to the OHI-S plaque index between community dwelling participants and nursing home residents with mild dementia (Tukey's HSD, $p < 0.0001$). Greater score indicates more plaque on the teeth.

A multiple linear regression indicated that after controlling for residency type the difference in plaque index is not explained by the significant difference in age (B = -0.009, CI = -0.043-0.025, p = 0.594). For every one-year increase in age, there is a decrease in plaque index by 0.009. Patients with dementia living in the nursing home are predicted to have a higher plaque index by a factor of 2.318 compared to a community dwelling patient with dementia of the sample age (**Figure 16**).

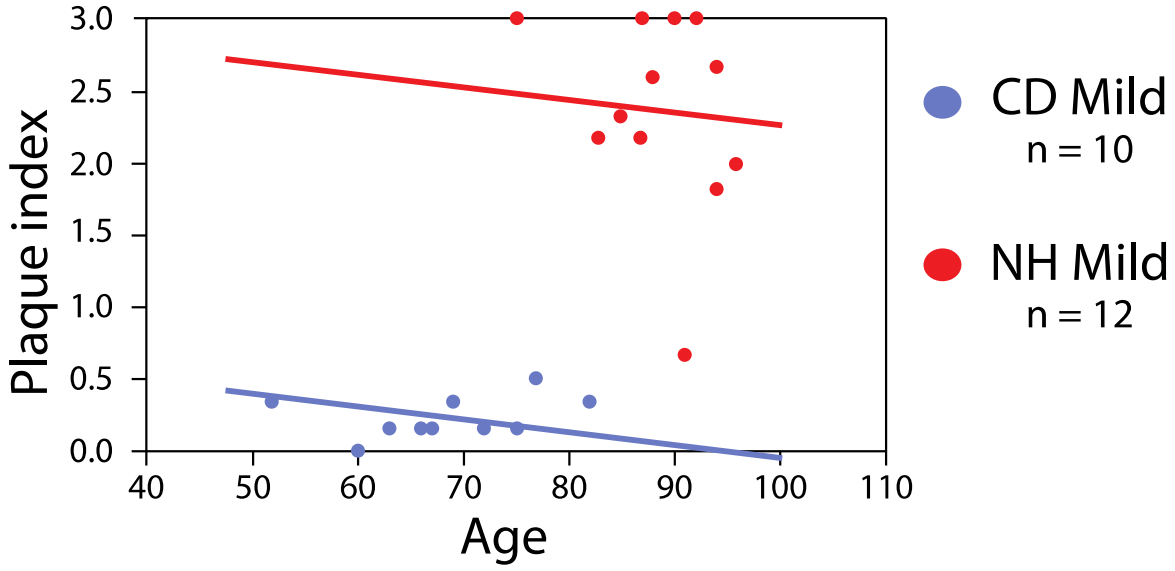


Figure 16: Regression of plaque index by age. Multiple linear regression shows that the difference in plaque index across residency type is not explained by a difference in age (B = -0.009, CI = -0.043-0.025, p = 0.594).

iii. Oral bacteria abundance and composition

Hard tissue samples were analyzed and compared between individuals in the community with mild dementia to nursing home residents with mild dementia. Alpha diversity did not significantly differ between the hard tissue samples of the groups (Kruskal-Wallis rank sum, p-Value = 0.9), indicating a similar number of species are found on the hard tissue across these groups (**Figure 17**).

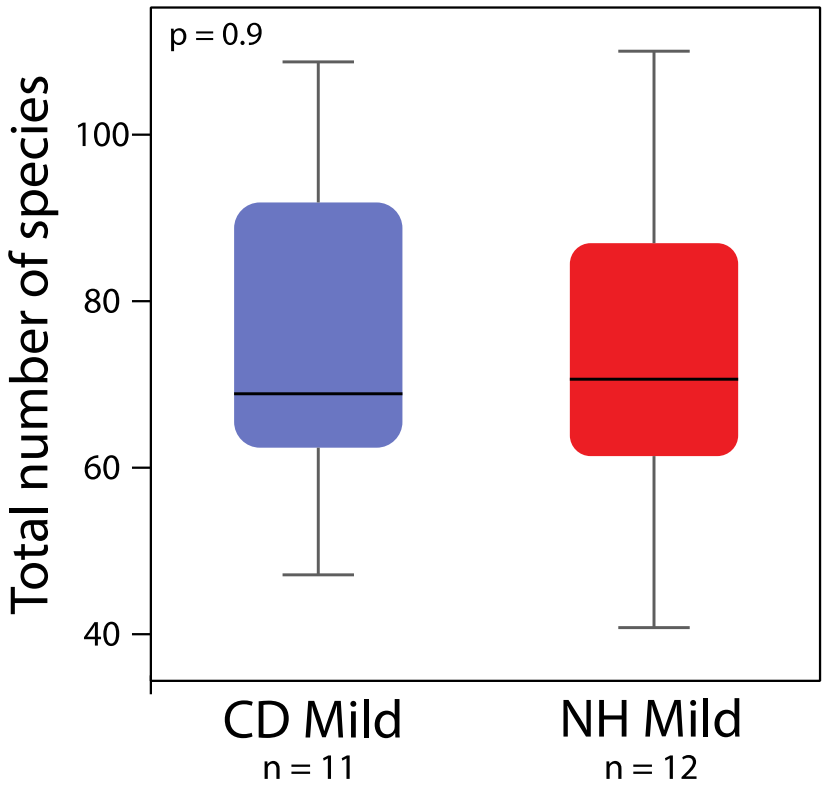


Figure 17: Alpha diversity of hard tissue samples of participants with mild dementia. Graph shows no difference in alpha diversity between groups (Kruskal-Wallis rank sum, p-Value = 0.9).

Soft tissue samples were analyzed and compared between individuals in the community with mild dementia to nursing home residents with mild dementia. Alpha diversity significantly differed

between the soft tissue samples of the groups (Kruskal-Wallis rank sum, p-Value = 0.03), indicating a different number of species are found on the soft tissue across these groups (**Figure 18**).

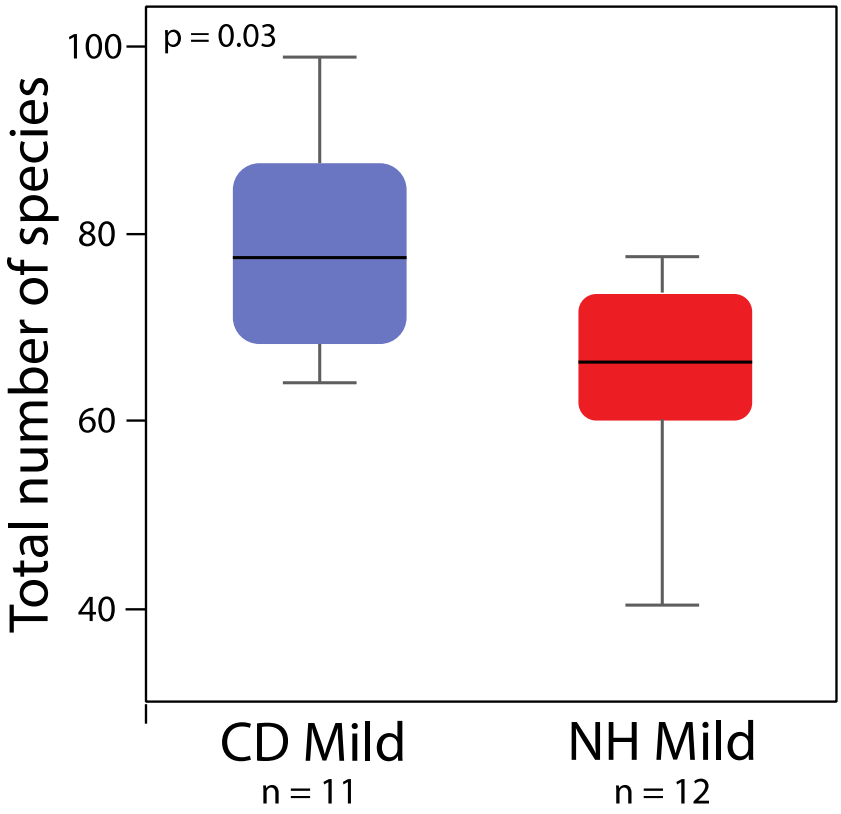


Figure 18: Alpha diversity of soft tissue samples of participants with mild dementia. Graph shows CD Mild subjects had a significantly greater mean number of species on the soft tissues compared to NH Mild subjects (Kruskal-Wallis rank sum, p-Value = 0.03).

Assessing for differences in bacteria composition between CD Mild and NH Mild groups' hard tissue samples, we found the beta diversity differed according to Weighted Unifrac (PERMANOVA, p=0.026). The beta diversity did not differ between CD Mild and NH Mild groups' hard tissue samples according to Bray Curtis distance (PERMANOVA, p = 0.129), Jaccard distance (PERMANOVA, p=0.107), and Unweighted Unifrac (PERMANOVA, p= 0.634) (**Figure 19A-D**).

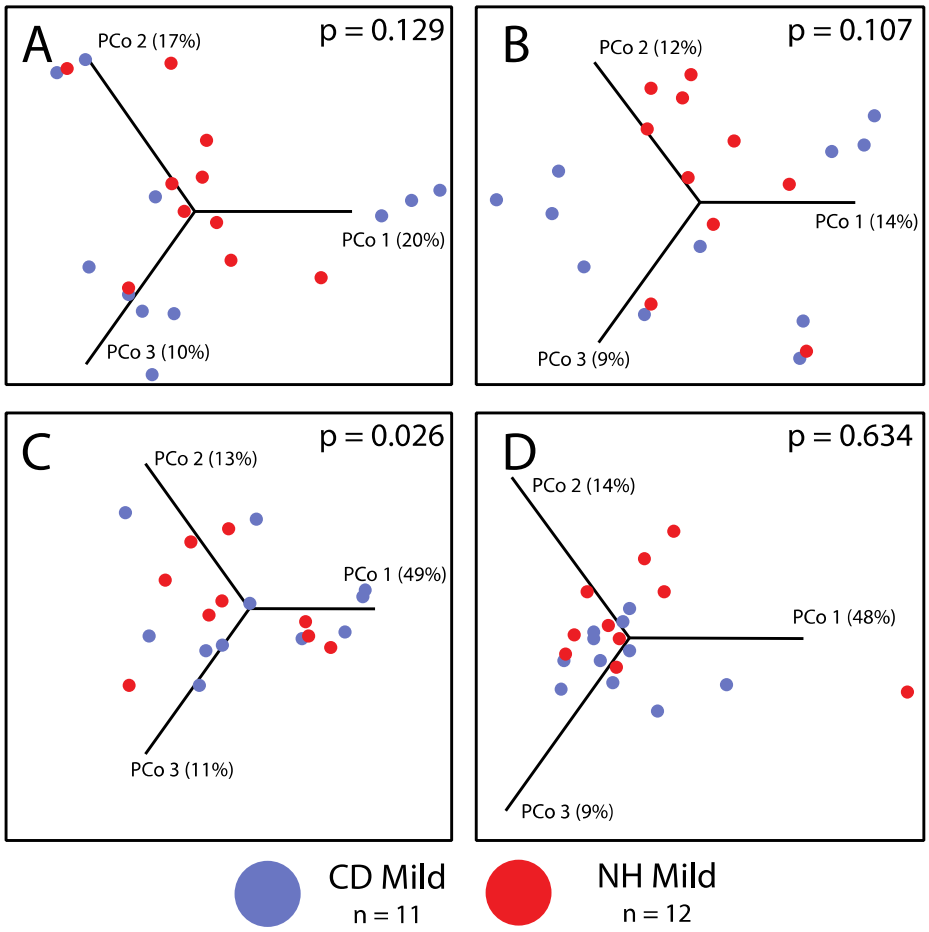


Figure 19: Beta diversity of the hard tissue samples between participants with mild dementia represented on PCo plots. Hard tissue samples from CD Mild and NH Mild subjects show no significant differences of bacterial communities according to (**A**) Bray Curtis distance (PERMANOVA, p = 0.129), (**B**) Jaccard distance (PERMANOVA, p=0.107), and (**D**) Unweighted Unifrac (PERMANOVA, p= 0.634). CD Mild and NH Mild subjects show significant differences in bacterial communities on the hard tissues according to (**C**) Weighted Unifrac (PERMANOVA, p=0.026).

We found that 63 species significantly differed between the CD Mild and NH Mild groups' hard tissue samples (**Figure 20**). Hard tissue samples from CD Mild participants had significantly

greater abundance of bacteria of the *Treponema* genus. Hard tissue samples from NH Mild participants had greater abundance of oral pathogenic bacteria of the genera *Actinomyces*, *Provetella*, *Lactobacillus*, and *Kingella*.

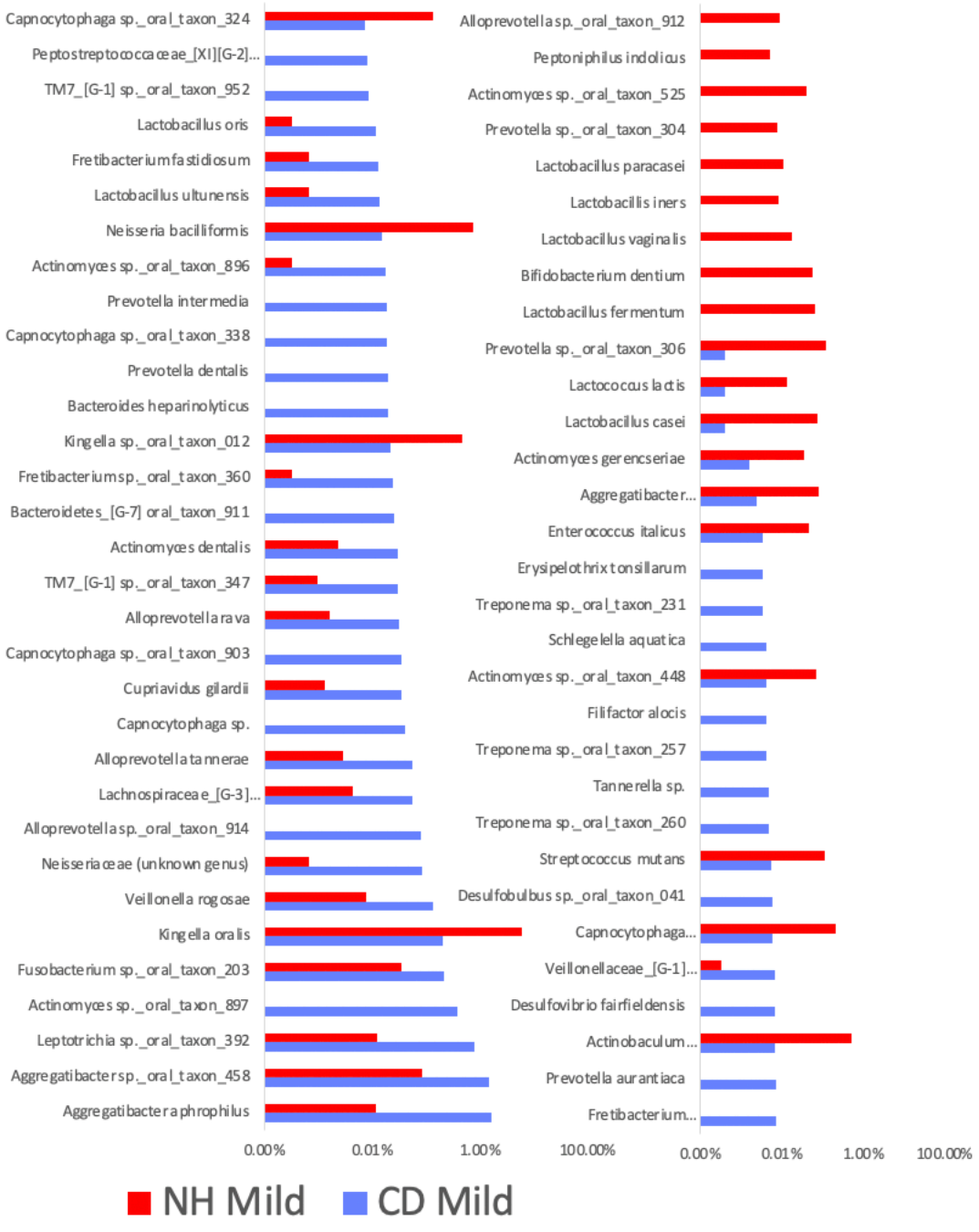


Figure 20: Relative abundance of bacteria that significantly differ on the hard tissues between participants with mild dementia. An abundance of 63 species significantly differed between the hard tissue samples of CD Mild and NH Mild participants.

Assessing for differences in bacteria composition between CD Mild and NH Mild groups' soft tissue samples, we found the beta diversity differed according to Bray Curtis distance (PERMANOVA, p = 0.03), Jaccard distance (PERMANOVA, p=0.016), and Weighted Unifrac (PERMANOVA, p= 0.017) (**Figure #A-C**). The beta diversity did not differ between CD Mild and NH Mild groups' soft tissue samples according to Unweighted Unifrac (PERMANOVA, p= 0.213) (**Figure #D**).

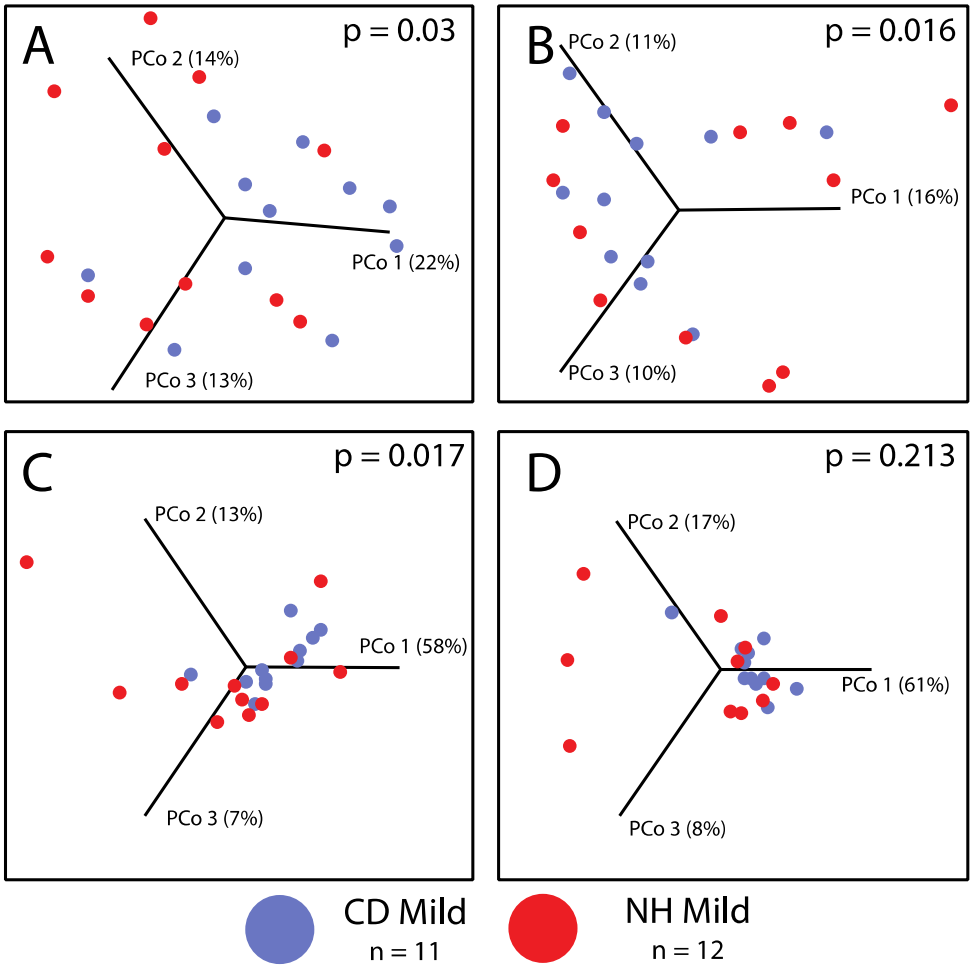


Figure 21: Beta diversity of the soft tissue samples between community dwelling and nursing home residents with mild dementia represented on PCo plots. Samples from CD Mild and NH Mild subjects show significant differences of bacterial communities on the soft tissues according to (**A**) Bray Curtis distance (PERMANOVA, p = 0.03), (**B**) Jaccard distance (PERMANOVA, p=0.016), and (**C**) Weighted Unifrac (PERMANOVA, p= 0.017). CD Mild and NH Mild subjects show no significant difference in bacterial communities on the soft tissues according to (**D**) Weighted Unifrac (PERMANOVA, p=0.213).

We found that 75 species significantly differed between the CD Mild and NH Mild groups' soft tissue samples (**Figure 20**). Soft tissue samples from NH Mild participants had greater abundance of oral pathogenic bacteria of the genera *Actinomyces, Provetella, Kingella,* and *Lactobacillu*s and significantly greater abundance of the species *Escherichia coli*.

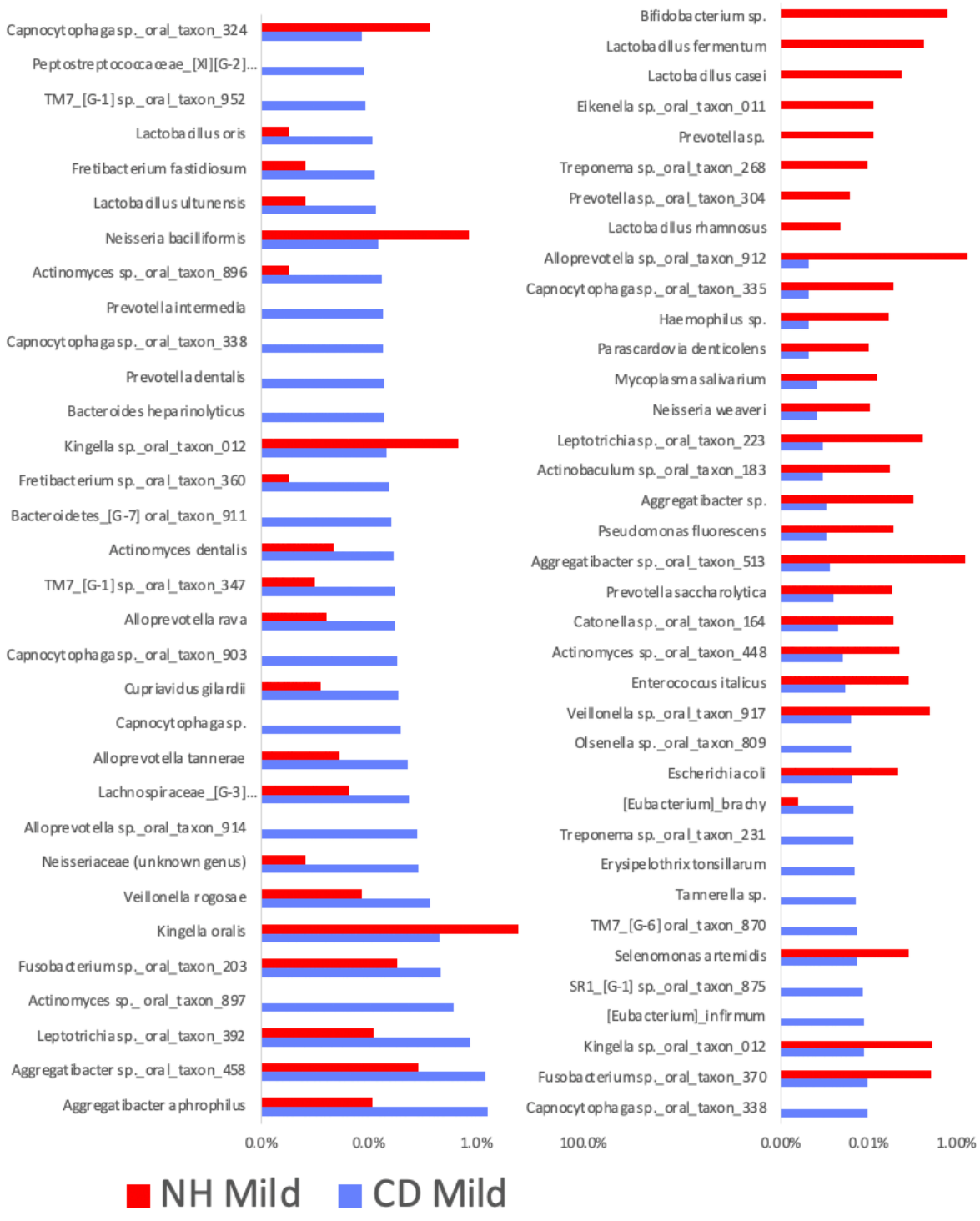

Capnocytophaga sp._oral_taxon_324
Peptostreptococcaceae_[XI][G-2]...
TM7_[G-1] sp._oral_taxon_952
Lactobacillus oris
Fretibacterium fastidiosum
Lactobacillus ultunensis
Neisseria bacilliformis
Actinomyces sp._oral_taxon_896
Prevotella intermedia
Capnocytophaga sp._oral_taxon_338
Prevotella dentalis
Bacteroides heparinolyticus
Kingella sp._oral_taxon_012
Fretibacterium sp._oral_taxon_360
Bacteroidetes_[G-7] oral_taxon_911
Actinomyces dentalis
TM7_[G-1] sp._oral_taxon_347
Alloprevotella rava
Capnocytophaga sp._oral_taxon_903
Cupriavidus gilardii
Capnocytophaga sp.
Alloprevotella tannerae
Lachnospiraceae_[G-3]...
Alloprevotella sp._oral_taxon_914
Neisseriaceae (unknown genus)
Veillonella rogosae
Kingella oralis
Fusobacterium sp._oral_taxon_203
Actinomyces sp._oral_taxon_897
Leptotrichia sp._oral_taxon_392
Aggregatibacter sp._oral_taxon_458
Aggregatibacter aphrophilus
0.0%
0.0%
1.0%
Bifidobacterium sp.
Lactobacillus fermentum
Lactobacillus casei
Eikenella sp._oral_taxon_011
Prevotella sp.
Treponema sp._oral_taxon_268
Prevotella sp._oral_taxon_304
Lactobacillus rhamnosus
Alloprevotella sp._oral_taxon_912
Capnocytophaga sp._oral_taxon_335
Haemophilus sp.
Parascardovia denticolens
Mycoplasma salivarium
Neisseria weaveri
Leptotrichia sp._oral_taxon_223
Actinobaculum sp._oral_taxon_183
Aggregatibacter sp.
Pseudomonas fluorescens
Aggregatibacter sp._oral_taxon_513
Prevotella saccharolytica
Catonella sp._oral_taxon_164
Actinomyces sp._oral_taxon_448
Enterococcus italicus
Veillonella sp._oral_taxon_917
Olsenella sp._oral_taxon_809
Escherichia coli
[Eubacterium]_brachy
Treponema sp._oral_taxon_231
Erysipelothrix tonsillarum
Tannerella sp.
TM7_[G-6] oral_taxon_870
Selenomonas artemidis
SR1_[G-1] sp._oral_taxon_875
[Eubacterium]_infirmum
Kingella sp._oral_taxon_012
Fusobacterium sp._oral_taxon_370
Capnocytophaga sp._oral_taxon_338
100.0%
0.00%
0.01%
1.00%
NH Mild
CD Mild

Figure 22: Relative abundance of bacteria that significantly differ on the soft tissue between participants with mild. An abundance of 75 species significantly differed between the soft tissue samples of CD Mild and NH Mild participants.

Oral health factors measured were not responsible for differences in oral health seen across residency type.

i. Oral hygiene habits

There were no differences in general oral hygiene habits measured by an oral hygiene questionnaire between community dwelling participants with mild dementia and nursing home residents with mild dementia. CD Mild and NH Mild subjects reported no differences in tooth brushing frequency (chi-square test of independence, χ^2 (2, N = 23 = 1.218, p = 0.544), flossing frequency (chi-square test of independence, χ^2 (2, N = 23) = 3.172, p = 0.205), and dental visit frequency (chi-square test of independence, χ^2 (2, N = 23) = 5.840, p = 0.054) (**Table 13**).

Table 13: Oral hygiene habits across residency type

	CD Mild (n = 11)	**NH Mild (n = 12)**	**p-value†**
Tooth brushing N (%)			0.544
Meets or exceeds daily recommendation (≥2x/day)	9 (82)	8 (67)	
Below recommendation	2 (18)	3 (25)	
Never	0 (0)	1 (08)	
Flossing N (%)			0.205
Meets or exceeds daily recommendation (≥1X/day)	6 (55)	4 (33)	
Below recommendation	4 (36)	3 (25)	
Never	1 (09)	5 (42)	
Dental visit frequency N (%)			0.156
Meets or exceeds yearly recommendation (≥2x/year)	8 (73)	3 (25)	
Below recommendation	3 (27)	7 (58)	
Never	0 (0)	2 (17)	

CD: Community dwelling, NH: Nursing home. † Chi-square calculated for categorical data.

ii. Assistance when performing oral hygiene tasks

There were no differences across residency type in assistance required to perform oral hygiene habits (chi-square test of independence, χ^2 (1, N = 30) = 2.008, p = 0.156) (**Table 14**).

Table 14: Requirement of assistance for oral hygiene across residency

	CD Mild (n = 11)	**NH Mild (n = 12)**	**p-value†**
Require assistance with oral hygiene tasks N (%)	0 (0)	2 (17)	0.156

CD: Community dwelling, NH: Nursing home. † Chi-square calculated for categorical data.

iii. Oral cleanliness

There were no differences in oral cleanliness based on food particle and tartar density in the oral cavity between community dwelling participants and nursing home residents with mild dementia (chi-square test of independence, χ^2 (2, N = 23) = 3.139, p = 0.208) (**Table 15**).

Table 15: Oral cleanliness across residency type

	CD Mild (n = 11)	**NH Mild (n = 12)**	**p-value**[†]
Food particles/tartar in N (%)			0.208
No places	5 (45)	6 (50)	
1-2 places	5 (45)	2 (17)	
≥3 places	1 (10)	4 (33)	

CD: Community dwelling, NH: Nursing home. [†] Chi-square calculated for categorical data.

iv. Dietary habits

Comparing across residency type, we found no subjects in either group required a modified, soft food diet (p-value = 1). Between the community dwelling and nursing home subjects with mild dementia, we found no differences in daily consumption of meals (chi-square test of independence, χ^2 (1, N = 23) = 1.552, p = 0.213), snacks (chi-square test of independence, χ^2 (2, N = 23) = 0.558, p = 0.757), sweets (chi-square test of independence, χ^2 (2, N = 23) = 1.168, p = 0.558), carbohydrates (chi-square test of independence, χ^2 (2, N = 23) = 3.916 0, p = 0.141), or sugary drinks (chi-square test of independence, χ^2 (2, N = 23) = 2.695, p = 0.260) (**Table 6**).

Table 16: Dietary habits questionnaire scores across residency type

	CD Mild (n = 11)	**NH Mild (n = 12)**	**p-value†**
Requires a modified, soft food diet (%)	0 (0)	0 (0)	1
Meals per day (%)			0.213
<3 meals/day	4 (36)	1 (8)	
3 meals/day	7 (64)	11 (92)	
>3 meals/day	0 (0)	0 (0)	
Snacks per day (%)			0.757
≤1 snack/day	7 (64)	8 (67)	
2 snacks/day	2 (18)	3 (25)	
≥3 snacks/day	2 (18)	1 (8)	
Sweet foods per day (%)			0.558
No sweets/day	4 (36)	3 (25)	
1-2 sweets/day	7 (64)	8 (67)	
≥3 sweets/day	0 (0)	1 (8)	
Carbs per day (%)			0.141
No carbs/day	2 (18)	4 (33)	
1-2 carb/day	6 (55)	8 (67)	
≥3 carbs/day	3 (27)	0 (0)	
Sugary drinks per day (%)			0.260
No drinks/day	6 (55)	3 (33)	
1-2 drink/day	4 (36)	8 (59)	
≥3 drinks/day	1 (09)	1 (8)	

CD: Community dwelling, NH: Nursing home. † Chi-square calculated for categorical data.

v. Salivary pH

There were no differences in salivary pH between community dwelling participants with mild dementia nursing home residents with mild dementia (Student's T-test, p = 0.384) (**Figure 23**).

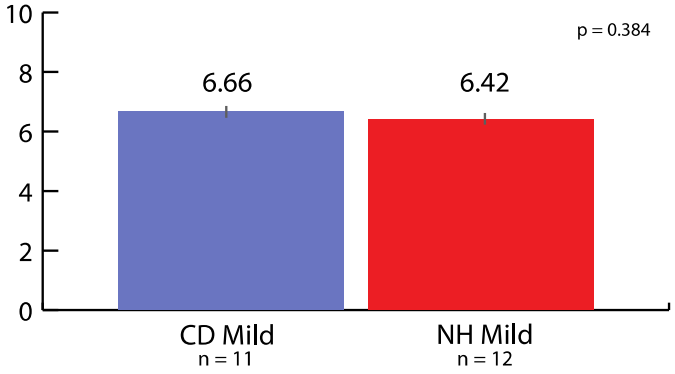


Figure 23: Salivary pH across residency type. Bar graph shows no difference of salivary pH between community dwelling participants and nursing home residents with mild dementia (Student's T-test, p = 0.384).

vi. Swallowing ability

There were no differences in swallowing ability between community dwelling participants with mild dementia and nursing home residents with mild dementia (chi-square test of independence, χ^2 (1, N = 23) = 0.004, p = 0.949) according to the RADBOUD Oral Motor Inventory (**Table 17**).

Table 17: RADBOUD swallowing ability across residency type

	CD Mild (n = 11)	**NH Mild (n = 12)**	**p-value**[†]
Swallowing difficulty (%)			0.949
None	11 (91)	11 (92)	
Mild	1 (9)	1 (8)	
Severe	0 (0)	0	

CD: Community dwelling, NH: Nursing home. [†] Chi-square calculated for categorical data.

CHAPTER 5: DISCUSSION

The overall goal of this study was to observe the oral health status of individuals with dementia and to determine factors affecting oral health that could lead to oral and systemic disease. My focus was to determine how oral health changes with level of dementia or residency type. The results from my research may allow individuals with dementia, and their loved ones, to prevent or slow further disease progression related to oral health. This study is significant as it is one of few to study both oral health and oral bacteria composition in systemic disease. This study adds clear evidence to a new and growing understanding that oral bacterial composition should be considered when evaluating oral health in patients with cognitive decline.

Oral health differences across cognitive status

One objective of this study is to determine how oral health changes with cognitive status. Understanding this change gives important information to families and their loved ones with dementia on what to expect as their cognitive decline progresses. We found no visual differences in oral health across cognitive status as there were no differences in the oral screening, number of natural teeth, chewing pairs, and plaque index across cognitive status. This finding of no visual differences does not agree with previous findings that patients with worse cognitive decline have greater rates of tooth decay, greater amounts of plaque, and greater tooth loss (Lee, 2019; Zhang, 2020; Ellefsen, 2007; Ide, 2016). We found that the bacterial abundance and composition did not differ when comparing subjects with mild dementia to controls, which does not agree with previous research (Wu, 2021). Though when comparing between subjects with mild and severe dementia, we found microscopic differences in oral health (differences in bacterial composition), which does agree with the few previous studies of the composition of oral bacteria in patients with dementia (Bathini, 2020; Cockburn, 2012). We found more pathogenic bacteria inhabit the oral cavity of those with severe cognitive decline. Though the number of species was not significantly different between subjects with mild and severe dementia, compared to those with mild dementia, patients with severe dementia had a greater abundance of opportunistic pathogenic bacteria on both their hard and soft tissues. Bacteria of the genera *Lactobacillus*, *Treponema*, and *Provotella* were found in greater abundance on both the hard and soft tissues of the nursing home severe dementia group. Bacteria of genera *Lactobacillus* and *Treponema* are known to cause tooth decay and periodontitis, respectively, which can lead to further oral disease (Caufield, 2015; Sela, 2001).

Not only were pathogenic oral bacteria found in greater abundance in subjects with severe dementia, but also bacteria that can exacerbate systemic disease. Bacteria of the genus *Treponema* have been studied for their possible involvement in the pathogenesis of AD, since they have been found in greater abundance of autopsy brain specimen of patients with AD compared to controls (Riviere, 2002). *In vivo* infection of mammalian cells with *Treponemes* lead to pathological features and hallmarks of AD (Miklossy, 2011). Some bacteria of the genus *Provotella* have caused respiratory infection (Larson, 2015). *Streptococcus pneumoniae* was also found in greater abundance on the soft tissues of the cavity in those in the nursing home severe dementia group. If *Prevotella* and *S. pneumoniae* are aspirated into the lungs, there is potential for fatal aspiration pneumonia, the leading cause of death of individuals with dementia.

Across cognitive status, we found no differences in oral hygiene habits, including frequency of tooth brushing, flossing, and dental visits, apart from subjects with severe dementia requiring significantly more assistance with oral hygiene tasks. We also found no difference in salivary pH or dietary habits, apart from significantly more subjects with severe dementia requiring a modified soft foods diet. We found that subjects with severe dementia had significantly worse ability to swallow when eating and taking pills.

The finding of no differences in oral health and factors affecting oral health suggests that a person with mild dementia is able to maintain their oral health and habits to protect their oral health. This suggest the cognitive decline experienced with mild dementia is not enough to cause the patients to forget to perform oral hygiene, change dietary preferences, and damage their ability to swallow, all of which allow their oral health to remain the same as controls. Difference in oral health between

subjects with mild and severe dementia suggests that disease progression begets worse oral health. The requirement of oral hygiene assistance, a soft food diet, and difficulty swallowing are the three oral health factors we found that are having a negative effect on the oral health of patients with severe dementia. Combined, these findings suggest that individuals with dementia and their caregivers should be very diligent in working to improve these oral health factors to prevent oral health decline.

A soft foods diet may explain the difference in the oral bacteria composition between the subjects with mild and severe dementia. Chewing hard/firm foods helps to remove plaque and tartar, which are both biofilms created by bacteria, from the teeth and gums. We also found that significantly more subjects with severe dementia require assistance when performing oral hygiene tasks compared to subjects with mild dementia. Though it was reported that all subjects requiring assistance with oral hygiene tasks did receive the needed assistance, these patients had a greater abundance of pathogenic oral bacteria, indicating assistance with oral hygiene is not as effective as by those who are able to perform the tasks themselves. Future studies are needed to evaluate the effectiveness of oral hygiene assistance, such as duration and what instruments are being used.

Across cognitive level, subjects with severe dementia had significantly worse swallowing ability, including while eating and taking pills, compared to subjects with mild dementia. Increased difficulty in swallowing ability means these subjects are more likely to aspirate food, liquids, and saliva into their lungs. Bacteria are also aspirated to the lungs. In health, bacteria do not remain in the lung for long, as healthy lungs are an inhospitable environment, boast close monitoring by the immune system, and have little nutrition for bacteria (Dickson, 2017). In aging populations and those

in poor health, pathogenic bacteria are more likely to flourish in the lungs and cause disease (Esme, 2019). The combination of pathogenic oral bacteria, especially respiratory pathogens, and swallowing difficulty in the subjects with severe dementia is extremely concerning, as these are the conditions that will lead to aspiration pneumonia, the number one cause of death of people with dementia (Brunnström, 2009). Our results and interpretations show interventions to improve bacterial composition and swallowing ability are gravely needed in this population.

Oral health differences across residency type

Another objective of this study was to determine if there are oral health differences between individuals with dementia that live in the community and those that live in nursing homes. To study this objective, we enrolled eleven participants with mild dementia living in the community and twelve participants with mild dementia living in one of two Houston-area nursing homes. Studying oral health across residency type is important because oral health status directly correlates with the prognosis of a patient with dementia. Thus, answering where a patient should live to best promote oral health is paramount to their long-term survival. Although there are many considerations involved when deciding to house a loved one in a nursing home, understanding how place of residence impacts oral health of patients with dementia can give families more information on which to base this very important decision.

Although there was no difference in overall oral health according to the total oral screening score, nursing home residents with mild dementia had significantly fewer chewing pairs compared to community dwelling patients with mild dementia. Effective mastication requires teeth to be paired from the top and bottom jaw. If a tooth does not have its opposing tooth to complete the pair, it becomes ineffective in proper chewing. The nursing home residents may be not getting all their required nutrients from food since mastication is the first step in the digestion process (El Helou, 2014; Sahyoun, 2003). Since tooth loss is a risk factor of cognitive decline, it is plausible that the nursing home residents' cognitive health may decline at a faster pace compared to the community dwelling participants with mild dementia; future longitudinal studies are needed.

Nursing home residents with mild dementia had significantly worse oral health according to the plaque index, our second measure of oral health. This means greater amounts of plaque reside on their teeth than on those who live in the community. Plaque is a biofilm of bacteria that produces acids. Over time, this erodes tooth enamel and leads to cavities and gum disease (Nazir, 2017; Albandar, 2000; Rathee, 2021). The significantly greater amounts of plaque on the teeth of the nursing home residents with dementia indicate likelihood that in time there will be differences in the oral screening as well. That oral health of nursing home residents is worse when compared to community dwelling participants agrees with previous studies that show fewer teeth and greater plaque in nursing home residents (Zimmerman, 2017; Porter, 2015; Kiyak, 1993; Frenkel, 2000).

We determined that neither age, nor any of the oral health factors measured (oral hygiene habits, oral cleanliness, oral hygiene assistance requirement, dietary habits, salivary pH, swallowing ability) was likely contributing to the difference in oral health according to the plaque index seen across residency type. This disagrees with previous research that shows nursing home residents are less likely to visit the dentist because they have greater difficulty in accessing oral health services (Chiesi et al., 2019), have poor oral hygiene habits (Sifuentes, 2020), and tend to have poor dietary habits (Pavlovic, 2019; Rodriguez-Rejon, 2019).

We also found differences in oral health across residency type according to our third measure of oral health – the oral microbiome. Oral bacteria abundance on the soft tissues and oral bacteria composition on the hard and soft tissues differed by place of residence. Community dwelling patients had an increase of bacteria of the genera *Treponema*, which have been thought to be involved in the pathogenesis of periodontal disease as well as AD (Miklossy, 2011). We found significantly more

oral disease-causing bacteria in the oral cavity of nursing home residents with mild dementia compared to community dwelling subjects with mild dementia. The bacteria that were in greater abundance in the nursing home residents with mild dementia included bacteria known to cause cavities and periodontal disease (Caufield, 2015; Sela, 2001). Bacteria of the genera, *Kingella* were also found in greater abundance in the oral cavity of nursing home residents with mild dementia compared to community dwelling participants with mild dementia. This bacteria genus is known to be associated with great abundances of plaque in the oral cavity (Yagupsky, 2012), which agrees with our findings that the nursing home residents have increased plaque. *E. coli* was found to be in greater abundance in the oral cavity of the nursing home residents. *E. coli* is known to create bacterial amyloid proteins that are thought to prime the host's immune system and cause neuronal amyloid production (Friedland, 2017). These findings agree with a 2018 study comparing oral bacteria across residency type by Ogawa et al. Other research has also found pathogenic bacteria in the oral cavities of patients residing in nursing homes but did not compare to subjects living in the community (Iwauchi, 2019; Kageyama, 2018), (Le, 2020).

Applications of these findings

Overall, our study has determined that a clinical/visual oral screening, which is standard practice, is not enough to determine the overall oral health of individuals with dementia. We cannot stop at the oral screening because even when there are no visual differences in oral health, microscopic differences exist in oral health across cognitive levels and across residency type. We recommend implementation of regular bacterial composition screening in patients with dementia in both the community and nursing homes since it is a non-invasive and relatively cheap procedure that gives important information on pathogenic bacteria that have great potential to lead to oral and systemic disease.

We also recommend paying attention to the quality of oral hygiene as dementia progresses and as patients with dementia transition to living in nursing facilities. Though we found no differences in the frequency of oral hygiene across cognitive ability and residency type, the differences in oral health across cognitive ability and residency type suggest more effective oral hygiene is needed to prevent further oral health decline. The differences in oral health between those with mild dementia and severe dementia suggests it is between these two cognitive levels that dementia hinders oral health and factors affecting oral health like the ability to perform oral hygiene and swallow properly. We suggest patients with dementia and caregivers of these patients focus on quality and effectiveness of oral hygiene, increasing frequency of oral hygiene as the disease progresses to combat the decline in oral health. Differences in oral health according to plaque index and oral bacteria composition across residency type indicate that better quality and more effective oral hygiene is needed to combat the dysbiosis of the oral bacterial composition and plaque buildup. Improving the quality of oral hygiene in nursing homes and increasing the frequency of dental visits

will help to decrease plaque on the teeth of nursing home residents with dementia. This intervention is key to prevent further oral and systemic disease.

Benefits of cross-sectional studies vs longitudinal studies

It is not yet known if oral health and the oral microbiome have an acute relationship with dementia, a chronic relationship with dementia or both. With such a large and broad question, there are benefits to studying oral health and the oral microbiome in patients with dementia in both longitudinal and cross-sectional studies. Our study provides a measure of acute effects of oral health and microbiome on dementia, as well as a measure of immediate risk of oral and systemic disease. Our study also shows that the oral microbiome is a loose biomarker of cognitive status. By design of the study, though, we cannot make any claims about causality of dementia by poor oral health or the oral microbiome. The benefits of a cross-sectional design include that we can determine immediate behavior changes - like more effective oral hygiene habits and interventions to help improve swallowing ability - that can easily be implemented to prevent further oral and systemic diseases in people currently living with dementia.

In addition to cross-sectional studies, longitudinal studies on the oral microbiome and dementia will also prove to be important to the field. Longitudinal studies will help parse out if and how oral bacteria are involved in disease pathogenesis of dementia. Longitudinal microbiome studies are becoming more feasible now as high throughput techniques of determining bacterial composition are more available and affordable to researchers. Longitudinal studies will allow the field to know how oral health and the oral microbiome change over time. If started early enough in the time course of a patient's life, researchers can determine if particular oral diseases or oral bacteria proceed dementia onset. A drawback to longitudinal studies is the long time course, which requires years of study follow up with results not immediately helping individual patients.

A model helping to explain the complex relationships between oral health, oral microbiome, and dementia

Much of the research done in the field of dementia is aimed at determining risk factors of dementia and when in a person's life these lead to cognitive decline. Based on literature review and meta-analyses, the Lancet Commission on Dementia placed 12 potentially modifiable risk factors on a life-course model based on when the factor increases risk for dementia (Lancet Commission on Dementia, 2020). Though thorough, their model does not include any oral health risk factors of dementia such as gum disease/tooth decay, tooth loss, and poor oral hygiene, all of which have been shown to be associated with cognitive decline (Beydoun, 2020; Qi, 2021; Thompsell, 2017).

The importance of a model of oral health risk factors of dementia is two-fold. The first is a direct benefit to individuals at any age who desire to prevent cognitive decline. With a better understanding of when these risk factors begin to impact cognitive decline, patients and professionals can attend to changing habits at or before key times in a patient's life-course to reduce the oral health risk factor's impact on potential future cognitive decline. Better understanding of these risk factors allows for great potential to delay or prevent dementia. The second reason this model is important is it has a direct benefit on the field of research, especially when it comes to oral bacteria. Since the field studying oral microbiome is relatively new, there are no longitudinal data that determine exactly when in life oral bacteria become a risk factor. This model can help direct future studies in the field of dementia, oral health, and oral microbiome as to when in the human life-course oral health and oral microbiome should be studied for its possible effects on future cognitive decline.

The Lancet Commission on Dementia categorizes the potentially modifiable risk factors into three age categories: early-life (<45 years of age), midlife (45-65 years of age), and old-age (>65 years of age). The Commission describes early-life factors as risk factors that affect cognitive reserve, or the ability of an individual and their brain to cope with pathology of neurological diseases, in this case dementia (Stern, 2009). The Commission describes less education as an early life risk factor. Midlife and old-age risk factors are described as risk factors that affect age related cognitive decline and trigger the pathological factors found in the brain. The risk factors the Commission lists as midlife risk factors include hearing loss, traumatic brain injury (TBI), hypertension (HTN), > 21 units of alcohol consumption, and obesity. Factors the Commission suggests begin acting as risk factors late in life include smoking, depression, social isolation, physical inactivity, air pollution, and diabetes. We will overlay on the Lancet model three evidence-based oral health risk factors at the key time in life when they are most impactful to cognitive health (**Figure 24**).

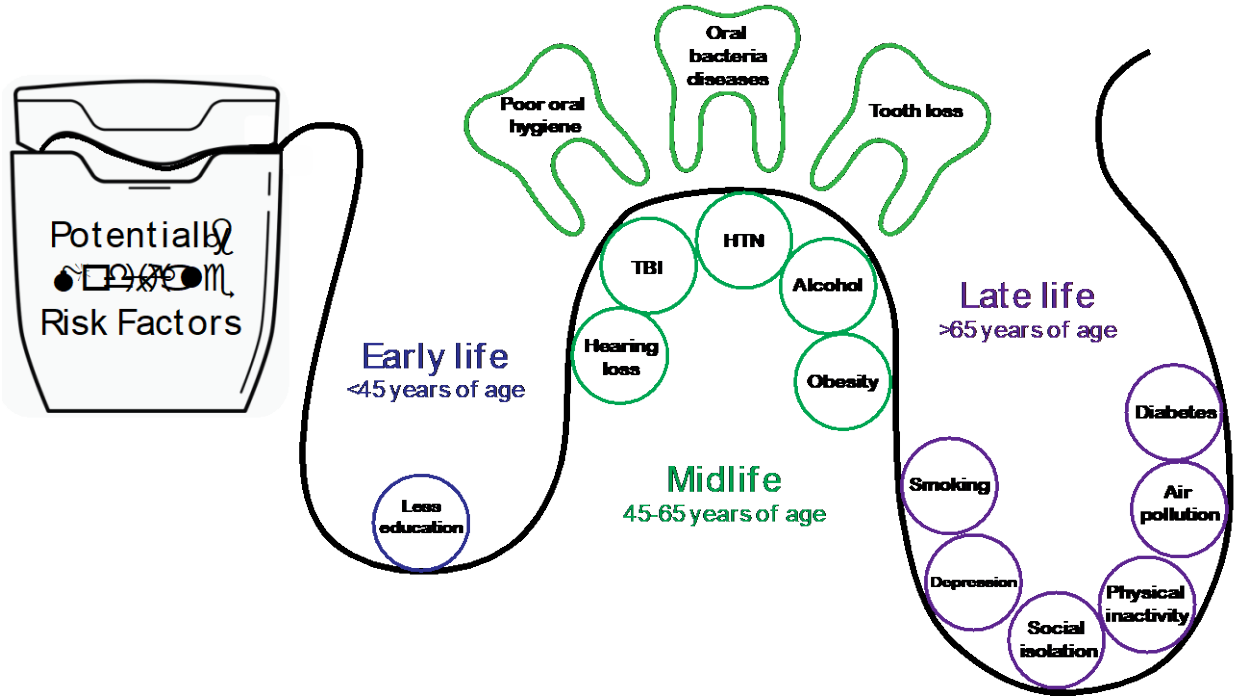


Figure 24: Oral health risk factor additions to the Lancet commission. Poor oral hygiene, oral bacteria diseases like dental caries and periodontal disease, and tooth loss should be considered risk factors of dementia beginning midlife, between the ages of 45-65. TBI: Traumatic brain injury, HTN: Hypertension.

The oral health risk factors we propose all fall into the categories of midlife risk factors as they are not involved in early-life cognitive reserve development. As research is showing, though, they are possibly involved in development of age-related cognitive decline and neuropathological features of dementia during the ages 45-65. The following paragraphs will discuss the three oral health risk factors (poor oral hygiene, oral infection, and tooth loss), how these act as risk factors of dementia, and at what time in life individuals should pay attention to these risk factors to prevent or delay future cognitive decline.

i. Oral hygiene

Good and proper oral hygiene is extremely important in the prevention of oral health problems. Because oral disease in adulthood has been shown to be involved in cognitive decline, it follows that oral hygiene should be just as important in the prevention and delay of cognitive decline. Zhang et al. discuss in their 2020 study of 102 participants that oral hygiene is a "relatively simple, inexpensive, and non-invasive approach for delaying cognitive decline." Without proper oral hygiene, plaque builds on teeth and calcifies into tartar within which opportunistic pathogenic bacteria flourish, which leads to cavities, periodontal disease and eventually tooth loss (Furuta, 2021; Baylum, 2007; Laudenbach, 2020; Pitts, 2017). Periodontal disease, tooth decay, and tooth loss can all be prevented with at-home and professional methods of oral hygiene (Heitz-Mayfield, 2002; Weijden, 2002; Hiremath, 2011; Prusty, 2021; Marchesan, 2020). Essentially, good oral hygiene can prevent poor oral health that otherwise leads to neurological damage and dementia later in life. Because of the research presented, we propose that poor oral hygiene is a modifiable risk factor of dementia that begins impacting cognitive health midlife. We propose that it begins impacting individuals as a risk factor of dementia prior to oral bacteria dysbiosis and tooth loss, since oral hygiene is important in preventing these oral health problems.

ii. Oral diseases caused by bacteria (caries and periodontal disease)

With high prevalence of oral diseases caused by bacteria, such as periodontal disease and caries, in the population of individuals with dementia and the growing field of research finding an association of oral bacteria and dementia, it is becoming apparent that oral bacteria and the resulting diseases should be considered a risk factor for cognitive decline. Data collected

between 2009-2014 for the National Health and Nutrition Examination Survey (NHANES) of 10,683 participants showed that 42.2% of all subjects had periodontal disease. Of the participants that were 30-44 years of age, only 29%, whereas 46% of participants aged 45-64 and 59.8% of participants aged >65 years had periodontal disease. Interestingly, the age group with the largest proportion of severe periodontal disease was those aged 45-64 years. The same survey found that a prevalence of 94.96% of participants aged 35-64 had cavities compared to 85.58% of adults aged 20-34 years of age (NHANES).

With this data, we propose oral diseases caused by bacteria should be considered a risk factor of dementia midlife. Preventing and slowing the growth of the pathogenic bacteria prior to it causing caries or periodontal disease, as well as, treating dental caries and periodontal disease early can prevent any acute inflammation from these diseases from becoming chronic inflammation. This is important to pay attention to because, as presented in the introduction, if not prevented or untreated, the inflammation that results from these oral infections cause chronic inflammation throughout the whole body including the brain, which may cause or exacerbate cognitive decline (Eisenstein, 2021).

iii. Tooth loss

According to previous research, tooth loss appears to become a risk factor in midlife, as this is when loss of permanent teeth frequently begins (NIH National Institute of Dental and Craniofacial Research, 2018). In a report by Dye et al. of the National Health and Nutrition Examination survey from 2011-2012, adults in the early life category were twice as likely to have lost no teeth (67%) compared to adults in the midlife time period (34%). As individuals age

into the old-age category, Dye et al. state tooth-retention is even lower with an estimated 19% of individuals older than 65 edentulous, having no remaining natural teeth. The survey found that the number of edentulous individuals doubled in those aged over 75 years compared to those aged 65-74 years old. Noticing and responding to the signs of tooth loss when an individual is in the midlife age category is a relatively simple approach to preventing and delaying future cognitive decline. We propose both oral bacteria dysbiosis and tooth loss to be risk factors beginning midlife, however we propose tooth loss becomes a risk factor after oral bacteria dysbiosis and the resulting diseases or periodontitis and caries, because of a majority of tooth loss in adulthood is caused by these diseases (NIH National Institute of Dental and Craniofacial Research, 2021; Junior, 2019; Mark, 2020).

Study limitations

i. Subjective questionnaires

To gather information on participants' oral hygiene habits, dietary habits, and swallowing ability, subjective questionnaires were utilized. Participants' answered questions presented by the researchers. This method allows for response bias, whether it is conscious or unconscious. There are more objective measures to measure oral hygiene habits, dietary habits, and swallowing ability that may have found different results than what our subjective questionnaires found, though these objective measures are more invasive, expensive, and take much more time from the researcher and the participant.

ii. Small sample size

With a total sample size of 52 subjects, 11 in the CD Control group, 11 in the MD Mild group, 12 in the NH Mild group, and 18 in the NH Severe group, our sample size for this study was relatively small. The small sample size makes it difficult to deem with strong confidence that our results are true or that the relationships in the data we see with the small sample size are what they would be in a larger population.

iii. All dementia types

Dementia is a broad term for cognitive decline from a previous baseline that interferes with daily activities and living. Dementia is not a disease itself; rather, it is a common disorder resulting from many diseases. Though all dementias have similar characteristics, the pathogenesis of different dementia causes diseases are not concretely known and may be

different. Studying multiple dementia types at once, as done in this study, may present a confounder as different dementia types may have different relationships with oral health.

iv. Lack of diversity of patient population

The diversity of the patient populations in this study is lacking with a large majority of the participants' race and ethnicity being white and non-Hispanic, respectively. A systematic review and meta-analysis published in 2021 by Shiekh et al. of 12 cohort studies and seven cross-sectional studies found that data suggest ethnic differences result in differing risk factors of dementia. Ethnic minorities are more likely to have the dementia risk factors of hypertension and diabetes and are more likely to have inequalities in care, likelihood of diagnosis and following medical treatment plans for dementia (Shiekh, 2021). It is because of these reasons studies of dementia and biomarkers of dementia take into consideration racial and ethnic differences amongst the population. This study cannot confidently determine if race or ethnicity are contributing factors in the oral health of patients with dementia.

Future studies in these populations

In this study, we measured oral health factors, which are factors that can change oral health status for the better or worse. We included oral hygiene habits, oral cleanliness, requirement of assistance with oral hygiene tasks, dietary habits, salivary pH, and swallowing ability. When comparing across residency type, we found no differences in the oral health factors, yet we did find differences in greater requirement of assistance, greater requirement of a modified soft foods diet, and greater difficulty in swallowing ability with increasing cognitive decline. Future studies should examine how these oral health factors are affecting the oral health, including oral bacterial composition.

Our study determined that across cognitive ability and across residency type, there were no differences in frequency of oral hygiene performed, however, we did not determine the duration for which these tasks are performed, nor if the performance of these tasks is effective. As well, we did find that there was a requirement of significantly more assistance with increasing cognitive decline but did not measure the time the assistance is given for, nor the effectiveness of the assistance. Oral hygiene tasks are maybe surprisingly technically difficult and require agility of the hands and mouth. Oral hygiene should be performed for at least 2 minutes at a time. Because of these considerations, future studies should measure the length of time spent on and effectiveness of oral hygiene tasks as these may explain differences in oral health across cognitive status and residency type that only noting the frequency of oral hygiene missed.

Because periodontal disease is strongly associated with cognitive decline, future studies of oral health and oral bacteria composition should additionally measure the prevalence and severity of

periodontal disease in the study subjects. Though our study did measure periodontal associated oral bacteria and amount of plaque which are risk factors of periodontal disease, the presence of these bacteria and plaque does not guarantee periodontal disease is present, so we cannot make any conclusions on the association of periodontal disease and dementia in the current study.

APPENDICES

APPENDIX A: KAYSER-JONES BRIEF ORAL HEALTH STATUS EXAMINATION (BOHSE)

Technician looks inside the mouth of the participant with the help of a mirror and small flashlight.

Category	Measurement	0	1	2
Lymph Nodes	Observe and feel nodes	☐ No enlargement	☐ Enlarged, not tender	☐ Enlarged and tender
Lips	Observe, feel tissue and ask patient, family or staff (e.g. primary caregiver)	☐ Smooth, pink and moist	☐ Dry, chapped or red at corners	☐ White or red patch; bleeding or ulcer for 2 weeks
Tongue	Observe, feel tissue and ask patient, family or staff (e.g. primary caregiver)	☐ Normal roughness, pink and moist	☐ Coated, smooth, patchy, severely fissured or some	☐ Red, smooth, white or red patch; ulcer for 2 weeks
Tissue inside cheek, floor and roof of mouth	Observe, feel tissue and ask patient, family or staff (e.g. primary caregiver)	☐ Pink and moist	☐ Dry, shiny, rough red, or swollen	☐ White or red patch, bleeding, hardness; ulcer for 2 weeks
Gums between teeth and/or under artificial teeth	Gently press gums with tip of tongue blade	☐ Pink, small indentations; firm, smooth and pink under artificial teeth	☐ Redness at border around 1-6 teeth; one red area or sore spot under artificial teeth	☐ Swollen or bleeding gums, redness at border around 7 or more teeth, loose teeth; generalized redness or sores under artificial teeth
Saliva (effect on tissue)	Touch tongue blade to center of tongue and floor of mouth	☐ Tissue moist, saliva free flowing and watery	☐ Tissues dry and sticky	☐ Tissues parched and red, no saliva
Condition of natural teeth	Observe and count number of decayed or broken teeth	☐ No decayed or broken teeth/roots	☐ 1-3 decayed or broken teeth/roots	☐ 4 or more decayed or broken teeth/roots; fewer than 4 teeth in either jaw
Condition of artificial teeth	Observe and ask patient, family or staff (e.g. primary caregiver)	☐ Unbroken teeth, worn most of the time	☐ 1 broken/missing tooth, or worn for eating or cosmetics only	☐ More than 1 broken or missing tooth, or either denture missing or never worn
Pairs of teeth in chewing position (natural or artificial)	Observe and count pairs of teeth in chewing position	☐ 12 or more pairs in chewing position	☐ 8-11 pairs of teeth in chewing position	☐ 0-7 pairs of teeth in chewing position
Oral cleanliness	Observe appearance of teeth or dentures	☐ Clean, no food particles/tartar in the mouth or on artificial dentures	☐ Food particles/tartar in one or two places in the mouth or on artificial teeth	☐ Food particles/ tartar in most places in the mouth or on artificial teeth

Total Score: _____

Upper dentures labeled: Yes ___ No ___ None___ Lower dentures labeled: Yes ___ No ___ None ___

Is your mouth comfortable? Yes _____ No _____ If no, explain:_____

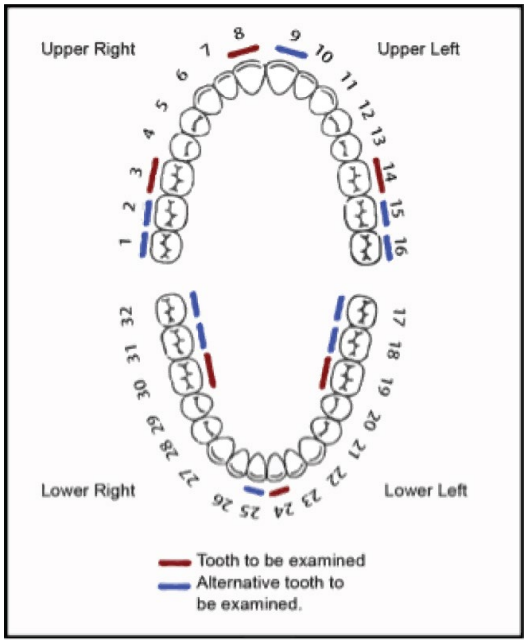
Number of remaining natural teeth: _____

Additional comments:

APPENDIX B: PLAQUE INDEX; SIMPLIFIED ORAL HYGIENE INDEX (OHI-S)

The Simplified Oral Hygiene Index (OHI-S) scores 6 of the tooth surfaces. The protocol for the Debris Index (DI-S) is based on numerical determinations representing the amount of debris found on the preselected tooth surfaces. This index will be calculated twice, once before and once after manual tooth brushing. **HurriView® Plaque Disclosing Snap -n-Go™** swabs are used to disclose plaque. The swabs are individually wrapped and prefilled with disclosing solution to highlight residual dental plaque pink. Swab teeth with plaque disclosing swab. Rinse gently, and score initial DI. Ask patient or caregiver to manually brush teeth the same way they do it at home (a toothbrush will be provided), then score DI again. Finally, if the DI remains high after tooth brushing, provide oral hygiene instructions.

SELECTION OF TOOTH SURFACES

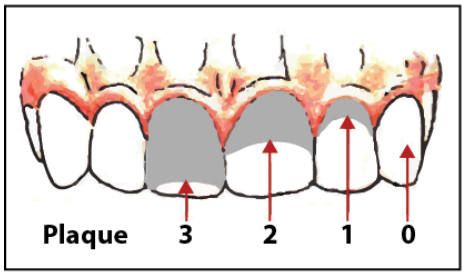


The six surfaces examined for the OHI-S are selected from four posterior and two anterior teeth.

- In the posterior portion of the dentition, the first fully erupted tooth distal to the second bicuspid (4, 13, 20, 29) usually the first molar (**3**, **14**, **19**, **30**) but sometimes (e.g., if tooth is missing) the second (2, 15, 18, 31) or third molar (1, 16, 17, 32), is examined. The buccal surfaces of the selected upper molars and the lingual surfaces of the selected lower molars are inspected.

- In the anterior portion of the mouth, the labial surfaces of the upper right (**8**) and the lower left central incisors (**24**) are scored. In the absence of either of these anterior teeth, the central incisor (9 or 25 respectively) on the opposite side of the midline is substituted.

CRITERIA FOR CLASSIFYING DEBRIS



Scores	Criteria
0	No debris or stain present.
1	Soft debris covering not more than one third of the tooth surface, or presence of extrinsic stains without other debris regardless of surface area covered.
2	Soft debris covering more than one third, but not more than two thirds, of the exposed tooth surface.
3	Soft debris covering more than two thirds of the exposed tooth surface.

EXAMPLE DI CALCULATION

After the scores for debris are recorded, the Index values are calculated. For each individual, the debris scores are totaled and divided by the number of surfaces scored. At least two of the six possible surfaces must have been examined for an individual score to be calculated. The average individual or group score is known as the Simplified Debris Index (DI-S). The DI-S values may range from 0 to 3. The following example shows how to calculate the index.

	Right molar		**Anterior**		**Left molar**		**Total**	
	Buccal	Lingual	Labial	Labial	Buccal	Lingual	Buccal	Lingual
Upper	3	-	2	-	3	-	8	-
Lower	-	2	-	1	-	2	1	4

Debris Index = (The buccal-scores) **+** (The lingual-scores) **/** (Total number of examined buccal and lingual surfaces).
Debris Index = (9+4) / 6 = 2.2

DEBRIS SCORE BEFORE TOOTH BRUSHING

	Right molar		**Anterior**		**Left molar**		**Total**	
	Buccal	Lingual	Labial	Labial	Buccal	Lingual	Buccal	Lingual
Upper	_____	_____	_____	_____	_____	_____	_____	_____
Lower	_____	_____	_____	_____	_____	_____	_____	_____

DI = (_____) + (_____) / (_____)

DI = _____

DEBRIS SCORE AFTER TOOTH BRUSHING

	Right molar		**Anterior**		**Left molar**		**Total**	
	Buccal	Lingual	Labial	Labial	Buccal	Lingual	Buccal	Lingual
Upper	_____	_____	_____	_____	_____	_____	_____	_____
Lower	_____	_____	_____	_____	_____	_____	_____	_____

DI = (_____) + (_____) / (_____)

DI = _____

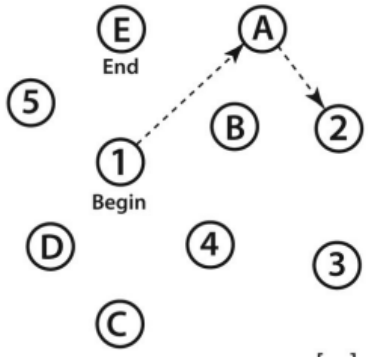
When was the last time you ate? _____
When was the last time you drank something other than water? _____
If either was within the last three hours, what did you eat or drink? _____

APPENDIX C: MONTREAL COGNITIVE ASSESSMENT (MOCA)

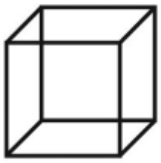
MONTREAL COGNITIVE ASSESSMENT (MOCA)
Version 7.1 Original Version

NAME :
Education : Date of birth :
Sex : DATE :

VISUOSPATIAL / EXECUTIVE — POINTS



Copy cube



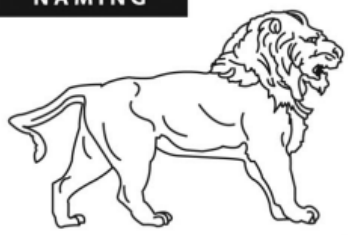
[ ] [ ]

Draw CLOCK (Ten past eleven)
(3 points)

[ ] Contour [ ] Numbers [ ] Hands

__/5

NAMING



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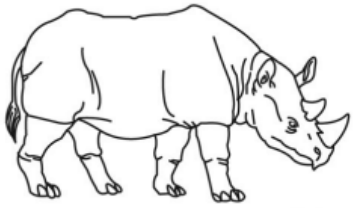
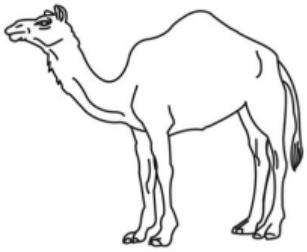


[ ]



[ ]

__/3

MEMORY Read list of words, subject must repeat them. Do 2 trials, even if 1st trial is successful. Do a recall after 5 minutes.

	FACE	VELVET	CHURCH	DAISY	RED
1st trial					
2nd trial					

No points

ATTENTION Read list of digits (1 digit/ sec.).

Subject has to repeat them in the forward order [ ] 2 1 8 5 4
Subject has to repeat them in the backward order [ ] 7 4 2

__/2

Read list of letters. The subject must tap with his hand at each letter A. No points if ≥ 2 errors
[ ] F B A C M N A A J K L B A F A K D E A A A J A M O F A A B

__/1

Serial 7 subtraction starting at 100 [ ] 93 [ ] 86 [ ] 79 [ ] 72 [ ] 65
4 or 5 correct subtractions: **3 pts**, 2 or 3 correct: **2 pts**, 1 correct: **1 pt**, 0 correct: **0 pt**

__/3

LANGUAGE Repeat : I only know that John is the one to help today. [ ]
The cat always hid under the couch when dogs were in the room. [ ]

__/2

Fluency / Name maximum number of words in one minute that begin with the letter F [ ] _____ (N ≥ 11 words)

__/1

ABSTRACTION Similarity between e.g. banana - orange = fruit [ ] train – bicycle [ ] watch - ruler

__/2

DELAYED RECALL

	Has to recall words **WITH NO CUE**	FACE [ ]	VELVET [ ]	CHURCH [ ]	DAISY [ ]	RED [ ]	Points for UNCUED recall only
Optional	Category cue						
	Multiple choice cue						

__/5

ORIENTATION [ ] Date [ ] Month [ ] Year [ ] Day [ ] Place [ ] City

__/6

© Z.Nasreddine MD **www.mocatest.org** Normal ≥ 26 / 30

TOTAL __/30
Add 1 point if ≤ 12 yr edu

Administered by: _____

APPENDIX D: ORAL HYGIENE HABITS QUESTIONNAIRE

Question	1 (Best)	2	3	4	5 (Worst)
How many times per day do you brush your teeth?	☐ 4 or more times a day.	☐ 3 times every day, after every meal.	☐ 2 times every day, usually morning and night.	☐ Once every day.	☐ Less than once every day.
How many times per day do you use dental floss to clean your teeth?	☐ 3 or more times a day.	☐ 2 times every day.	☐ Once every day.	☐ Only when I feel something in between my teeth.	☐ Never.
How often do you use mouthwash to clean or freshen your	☐ 2 or more times a day.	☐ 2 times every day.	☐ Once every day.	☐ Only when I feel I have bad breath.	☐ Never.
How much assistance do you need to brush and clean your teeth?	☐ None; I can do it by myself.	☐ Some; provided by a family member or caregiver.	☐ Complete; provided by a family member or caregiver.	☐ Some or complete; but I do not have anyone to assist me.	☐ My teeth do not get brushed or cleaned.
What type of toothbrush do you use?	☐ Electronic toothbrush exclusively.	☐ Electronic toothbrush most of the time.	☐ Electronic toothbrush occasionally.	☐ Manual toothbrush exclusively.	☐ I do not brush my teeth.
How often do you change your toothbrush?	☐ 4 or more times a year.	☐ 3 times a year.	☐ 2 times a year.	☐ Once every year.	☐ Never.
How often do you visit the dentist?	☐ 4 or more times a year.	☐ 3 times a year.	☐ 2 times a year.	☐ Once every year.	☐ Never.

Oral Hygiene Habits Questionnaire

Column Score: _____ _____ _____ _____ _____

Total Score: _____

APPENDIX E: DIETARY HABITS QUESTIONNAIRE

Question	1 (Best)	2	3	4	5 (Worst)
What kinds of foods are you able to eat?	☐ Any type of food whether it is hard or soft. Including things like raw carrots, apples or beef.	☐ Mostly hard foods.	☐ Mostly soft foods.	☐ Soft foods and liquid shakes.	☐ I am on a liquid diet.
How many main meals do you eat per day?	☐ 5 or more main meals per day.	☐ 4 main meals per day.	☐ 3 main meals per day	☐ 2 main meals per day.	☐ 1 main meal per day.
How many snacks do you eat per day?	☐ More than 4 snacks per day.	☐ 4 snacks per day.	☐ 3 snacks per day.	☐ 2 snacks per day.	☐ 1 or less snacks per day.
Of your meals and snacks, how many are sweets (sugary or desserts)?	☐ Never.	☐ 1 time per day.	☐ 2 times per day.	☐ 3 times per day.	☐ More than 3 times per day.
Of your meals and snacks, how many are breads, pastas, or rice?	☐ Never.	☐ 1 time per day.	☐ 2 times per day.	☐ 3 times per day.	☐ More than 3 times per day.
How many carbonated (sodas) **or sugar containing drinks** (coffee or tea with sugar) **do you drink per day?**	☐ Never.	☐ 1 time per day.	☐ 2 times per day.	☐ 3 times per day.	☐ More than 3 times per day.

Column Score: _____ _____ _____ _____

_____

Total Score: _____

APPENDIX F: RADBOUD ORAL MOTOR INVENTORY

Question	1 (Best)	2	3	4	5 (Worst)
What kinds of foods are you able to eat?	☐ Any type of food whether it is hard or soft. Including things like raw carrots, apples or beef.	☐ Mostly hard foods.	☐ Mostly soft foods.	☐ Soft foods and liquid shakes.	☐ I am on a liquid diet.
How many main meals do you eat per day?	☐ 5 or more main meals per day.	☐ 4 main meals per day.	☐ 3 main meals per day	☐ 2 main meals per day.	☐ 1 main meal per day.
How many snacks do you eat per day?	☐ More than 4 snacks per day.	☐ 4 snacks per day.	☐ 3 snacks per day.	☐ 2 snacks per day.	☐ 1 or less snacks per day.
Of your meals and snacks, how many are sweets (sugary or desserts)?	☐ Never.	☐ 1 time per day.	☐ 2 times per day.	☐ 3 times per day.	☐ More than 3 times per day.
Of your meals and snacks, how many are breads, pastas, or rice?	☐ Never.	☐ 1 time per day.	☐ 2 times per day.	☐ 3 times per day.	☐ More than 3 times per day.
How many carbonated (sodas) **or sugar containing drinks** (coffee or tea with sugar) **do you drink per day?**	☐ Never.	☐ 1 time per day.	☐ 2 times per day.	☐ 3 times per day.	☐ More than times per day.

Column Score: _____ _____ _____ _____

_____

Total Score: _____

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VITA

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