THE CORRELATION OF PERIODONTAL DISEASE AND CHRONIC ILLNESS WITHIN MODERN SKELETAL POPULATIONS

by

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DEDICATION

In Loving Memory of Maria Gallegos, my amazing grandmother who was always proud of my accomplishments.

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1. INTRODUCTION

Chronic diseases are widespread and are increasing in severity and prevalence within the United States (Aschner et al., 2014; Raghupathi & Raghupathi, 2018). In America, approximately 45% of the population reported having an autoimmune disease in the year 2000, with 61 million of those individuals having multiple chronic diseases (Anderson and Horvath, 2004). Not only do these diseases cause physical stress on their hosts, they also cause financial stress with 78% of health spending through healthcare dedicated to individuals with chronic conditions in America (Anderson and Horvath, 2004).

Periodontal Disease

The American disease experience could potentially explain the association found between periodontitis (also known as periodontal disease) and other chronic and inflammatory diseases. Periodontal disease has strong correlations with both chronic disease and stress (Aschner et al., 2014; Belstrøm et al., 2012; Dominy et al., 2019; Madianos and Koromantzos, 2018; DeWitte and Bekvalac 2011; DeWitte 2012; Djuric et al., 2009; Geber and Murphy 2018; Oyamada et al. 2010). Clinical dental and medical research has shown a strong correlation between periodontal disease and chronic and autoimmune diseases such as Alzheimer's disease, diabetes, and cardiovascular disease (CVD) to name a few (Aschner et al., 2014; Belstrøm et al., 2012; Dominy et al., 2019; Madianos and Koromantzos, 2018; Page and Eke, 2007). Anthropological research suggests that the presence of periodontal disease correlates with multiple external stressors (DeWitte and Bekvalac 2011; DeWitte 2012; Djuric et al., 2009; Geber and

Murphy 2018; Oyamada et al. 2010). Unfortunately, bioarchaeological research is often constrained to archaeological samples with limited to no documented information on demographic profiles or health status.

Defining Periodontitis, Risk Factors, and Stress

Periodontal disease is a bacterial infection which causes chronic inflammation within the supporting gingival soft tissues around the teeth. Periodontitis is a very complex disease due to the rates of deterioration determined at the individual level, in addition to general alveolar bone loss (Lindhe and Lang, 2015). This unique individuality in rates of deterioration is caused by many influential factors which have been known to cause periodontal disease, including genetics, demographics, stress, lifestyle habits (i.e., oral hygiene, smoking, alcohol consumption and diet), medications, and even hormonal changes within women (Nazir, 2017).

These influential factors that affect periodontal health will be referred to in this research as "risk factors." This is because both the modifiable factors (e.g. smoking, drinking) and non-modifiable factors (e.g., sex, race) increase the risk of developing periodontal disease (DeWitte, 2012; Geber and Murphy, 2019; Nazir, 2017; Naorungroj et al., 2017). These risk factors will be considered as potential contributors to stress that can lead to periodontal disease, resulting from biological, social, environmental, habitual, or health-related tension (Nazir, 2017). For the sake of this research, "stress" will be defined as "physical, chemical, or emotional factors that causes bodily or mental tension and may be a factor for disease causation" (Merriam-Webster, n.d.).

Clinical vs Anthropological Perspectives

Within modern populations, epidemiologists have found correlations between severity of periodontal disease with cardiovascular disease (CVD), hypertension, and diabetes (Nazir, 2017). However, research on correlations between periodontal disease and CVD, and the severity of expression of periodontal disease, has not been assessed in skeletal remains by anthropological practitioners. This study aims to improve our understanding of how chronic disease and stress correlate with alveolar bone loss in individuals with periodontal disease. In addition, another objective is to identify whether adjustments should be made toward current anthropological methods for estimating periodontal disease. Finally, this research seeks to compare two modern populations, one documented and one undocumented, to identify if periodontal severity can help further the understanding of stress between and among populations. In summary, this project will seek to bridge the clinical and anthropological perspectives on periodontal disease and chronic health conditions via the examination of oral health in a modern skeletal collection with documented medical data.

Literature Review

Biological anthropologists have established correlations in archaeological and historic populations with periodontal disease in terms of demographic factors such as age, sex and lifestyle that play a key role in the development of periodontal disease. For example, a greater prevalence of periodontal disease is present in males than females (DeWitte, 2012). Oral hygiene, diet and overall lifestyle play significant roles in the presence of periodontal disease resulting from socioeconomic class, smoking habit and

environment (Geber and Murphy, 2018; Oyamada et al., 2010). The anthropological assessment of periodontal disease using presence/absence scoring has been useful in identifying correlations with chronic disease and stress (Aschner et al., 2014; Belstrøm et al., 2012; DeWitte and Bekvalac 2011; DeWitte 2012; Djuric et al., 2009; Dominy et al., 2019; Geber and Murphy 2018; Madianos and Koromantzos, 2018; Oyamada et al. 2010; Page and Eke, 2007).

In order to understand these correlations further, in addition to presence/absence data, the *severity* of periodontal disease should also be investigated. Assessing the stage or progression of periodontal disease within skeletal cases was previously done by Djuric et al. (2009). However, their parameters for classification of early, moderate and severe periodontal disease were not quantified, and thus were difficult to replicate. Instead, the rank parameters were assessed by qualitative traits using morphology rather than metric analysis (Djuric et al., 2009).

The age of onset and rate in which periodontal disease deteriorates alveolar bone is variable from person to person due to the individual's health (i.e. chronic diseases), genetics, diet and oral hygiene (Nazir, 2017). The same factors can affect bone loss over time. Within clinical periodontology, the diagnosis of periodontal disease is assessed by taking measurements of pockets that form within the gingiva, alveolar bone loss, and loss of attachment of fibers and periodontal ligaments (Page and Eke, 2007). However, within the realm of skeletal periodontal evaluation in biological anthropology, these methods are often not applicable due to the absence of soft tissue structures in the mouth. This complicates the study of periodontal disease in skeletal populations and has limited the analysis of periodontal disease to mere presence or absence for over 30 years (Alfonso-

Durruty et al., 2014; Clarke et al., 1986; DeWitte and Bekvalak, 2011; Kinaston et al., 2019). While the presence and absence of periodontal disease can be useful in identifying larger environmental or societal stresses within a population, *severity* of periodontal disease has the potential to identify stress levels on an individual level. When comparing smaller groups within the same population, severity could conceivably identify which groups and subgroups were exposed to more demographic, social, environmental and health stress within a population (Cengiz et al., 2018; Eke et al., 2012; Geber and Murphy, 2018; Nazir, 2017; Oyamada et al., 2010).

The Link Between Chronic Disease and Periodontal Disease

Periodontal disease is an important factor in the correlation of chronic diseases such as cardiovascular disease (CVD), hypertension and diabetes (Belstrøm et al., 2012; Madianos and Koromantzos, 2018) as well as individual health. Studies have identified a low to moderate correlation between periodontal disease and CVD (Belstrøm et al., 2012; Fröhlich et al. 2016). However, some complications of these studies include the lack of universal definitions for periodontal disease and CVD, variations in sample sizes, and a lack of consensus in parameters for studies on CVD and periodontal disease (Belstrøm et al., 2012). Despite these complications, there has been a consistent correlation between CVD and periodontal disease (Belstrøm et al., 2012; Fröhlich et al., 2016).

A similar correlation can be seen with other chronic diseases such as diabetes.

Studies have shown that successful treatment of periodontal disease within three months of diagnosis shows a significant reduction in HbA1C (glycated hemoglobin) and after six

months of treatment the reduction of HbA1C is at a much lower still (Madianos and Koromantzos, 2018).

The field of epidemiology continually finds correlations between severity of periodontal disease and CVD, hypertension, and diabetes. Many current studies are focused on the relationships of periodontal disease inflammation and inflammatory pathways and the common features of inflammation in other systemic diseases (Linden, GJ et al., 2013; Schenkein, HA & Loos, BG, 2013; Taylor, JJ et al., 2013; Tonetti, MS & Van Dyke, TE, 2013; Van Dyke, TE & Winkelhoff, AJv, 2013). Epidemiologists state that heart disease accounts for 30% of mortality in America (Belstrøm et al., 2012), while diabetes afflicts over 24 million adults in Central and South America alone (Aschner et al., 2014). If anthropologists could explore the correlation between the severity of periodontal disease and chronic diseases, they could investigate correlations between chronic disease and bone loss in archaeological, historic, and modern skeletal remains.

In the following chapter, I will provide descriptive details of the samples and materials used within this study. In addition, I will provide a detailed layout of the methodology incorporated in this project.

Goals and Research Questions

The goal of this project is to fill in the gaps within the anthropological research literature to address the comorbidity of periodontal disease with other chronic disease states. Additionally, this study seeks to explore the potential benefits of utilizing an alternative measurement method for assessing periodontal disease. This includes the ability to compare populations. My research seeks to answer questions related to

periodontal disease within skeletal populations, as well as the effect of chronic disease on periodontal severity:

- (1) Does the presence of periodontal disease correlate with chronic illnesses in a medically documented modern skeletal sample and if so, does the severity of periodontal disease predict the severity of chronic diseases?
- (2) Does an adjusted periodontal measurement method, which assesses both presence or absence as well as severity, provide more accurate and explanatory information than the standard anthropological assessment that uses only presence/absence data?
- (3) What can differences in periodontal expression, severity, and distribution inform us about the health of unidentified individuals in undocumented skeletal populations?

The OpID sample was utilized to assess if the severity of periodontal disease distribution can identify similarities or discrepancies between the migrant populations and those from the TXSTDSC.

2. MATERIALS AND METHODS

Sample

This study examined non-edentulous mandibles and maxillae from 74 individuals from the Texas State Donated Skeletal Collection (TXSTDSC). All individuals were assessed for number of teeth available and condition of dentition. The overall sample had an age range of 18-91 with a bulk of the sample with a median age of 56 and an average age of 54 (Table 1). The TXSTDSC is a modern willed-body donated American skeletal sample that has demographic and medical information available provided by the donor, or their legal next of kin. The associated medical information provided the data for variables such as chronic disease (i.e., diabetes, cardiovascular disease, and hypertension), lifestyle (i.e., smoking, drinking), and the demographic data provided childhood socioeconomic status (SES), sex, age, and race/ethnicity. These are all variables that will be assessed for a correlation in both presence, absence, and severity of periodontal disease. The health and demographic data were not visible to the researcher during the data collection process.

<u>Table 1.</u> TXSTDSC age distribution table.

Age range	# of individuals
Under 30	7
30-39	6
40-49	10
50-59	26
60-69	15
70+	10

<u>Table 2.</u> Distribution of the TXSTDSC sample by ancestry and sex.

TXSTDSC Sample			
Ancestry	Males	Females	Total
White	44	24	68
Black	2	0	2
Hispanic	0	2	2
Other	1	0	1
Total	47	26	73

This study also examined individuals recovered by Operation Identification (OpID), an initiative under the direction of Dr. Kate Spradley of Texas State University that locates, identifies and repatriates the remains of individuals found along the US-Mexico border in south Texas. The OpID individuals are presumed migrant individuals who are reasonably assumed to be predominantly Central American (Spradley, 2016), although there are certainly individuals from neighboring geographic regions as well. For the OpID sample 50 individuals out of the 236 currently recovered were utilized. Since these individuals are unidentified, there is no demographic or medical information available. Instead, the estimated biological profiles were used as predicted or suspected demographics (see Table 3). This adjustment simulates the typical circumstances of discovering unidentified skeletal remains in both a medicolegal and bioarcheological context.

<u>Table 3.</u> Distribution of the Operation Identification sample by estimated sex and ancestry.

Operation Identification Sample					
Ancestry Males Females Unknown Total				Total	
Hispanic	17	15	0	32	
Unknown	12	3	3	18	
Total	29	18	3	50	

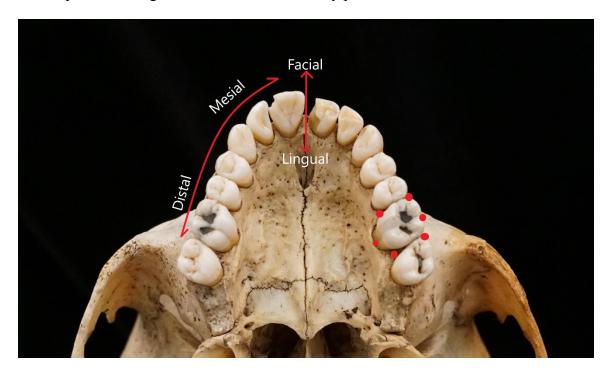
All individuals used in this study met the following criteria. Each individual had to have a minimum of four teeth per each of the four quadrants of the mouth. Of those four teeth, at least two had to be adjacent to each other. Additionally, teeth with enamel defects, or damage that affected the cementoenamel junction (CEJ) or the alveolar crest (AC) measurement were excluded from this study. Enamel defects do not include cervical enamel projections, an extended projection of enamel from the CEJ (Edgar, 2017; Turner 1991). However, measurements including cervical enamel projections were adjusted slightly coronally from the apex to get an accurate CEJ to AC measurement (James P. Fancher, personal communication, 2018).



<u>Figure 1.</u> A representation of the four quadrants of the mouth corresponding to upper right, upper left and lower left, lower right. Images from Donor 2012.036 from the TXSTDSC.

Procedure

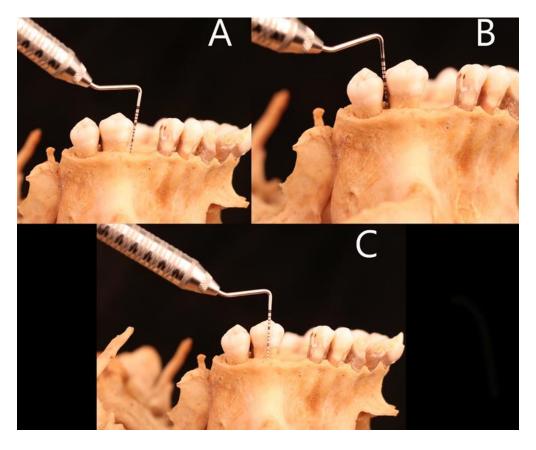
Prior to taking measurements, the skull was laid out and any loose teeth were reinserted into the alveolar bone. Some teeth did not fit back into their alveolar socket and were therefore excluded from the measurement to avoid damaging the alveolar crest. Afterwards, measurements were taken three times each for the first 15 individuals selected. These individuals were measured three times each to assess the interobserver error and standard deviation of the periodontal measurements. The rest of the sample was measured once. The demographic information and estimated biological profile was not known prior to taking measurements, to avoid any potential bias.



<u>Figure 2</u>. An image of donor 2012.036 that demonstrates both the anatomical nomenclature (left) and the six points of measurement (red circles)

The study of both the TXSTDSC and the OpID individuals utilized an adaptation of the standard clinical method to measure the distance between the cementoenamel junction (CEJ) to the alveolar crest (AC) (Gargiulo et al., 1961; Papapanou et al., 2018;

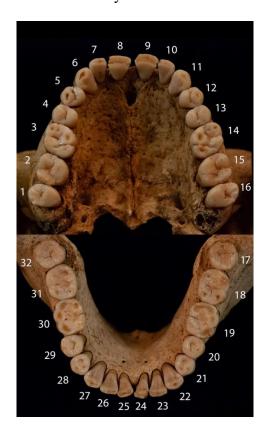
Tonetti et al., 2018; Page & Eke, 2007). These measurements were taken at 6 points for each present tooth (Figure 2) (Page & Eke, 2007; Eke et al, 2018). The measurements consisted of mesial, apical and distal measurements from both the facial and lingual sides of each tooth that corresponds to a Full Mouth Periodontal Exam as described in current periodontal literature (Figure 3) (Eke et al., 2018). Prior to data collection the researcher was trained and standardized in taking measurements by a very experienced board-certified periodontist.



<u>Figure 3.</u> Demonstrating the different positions of periodontal measurements from the facial, or buccal, position. A) shows a mesial measurement, B) shows a distal measurement, and C) shows an apical measurement.

For this project, I utilized a University of North Carolina (UNC) periodontal probe. The UNC periodontal probe has a line for every millimeter (mm) and was the easiest to read. Any measurement that was between periodontal lines was rounded to the

nearest mm (over half rounded up, below half rounded down). The assistance of a magnifying glass and headlamp was also used. For this study a Magnipro headset was used because it has both a light, and an assortment of magnification options. These measurements were initially collected on a periodontal diagnosis and treatment plan form created for the United States Air Force (see Appendix 1). The layout for this treatment plan made it important to utilize the universal numbering system (Figure 4) to create a system for taking measurements. This helped avoid and catch any transcription errors. For this study the teeth were measured right to left for both the maxilla and mandible, however this is strictly up to preference of the measurer/transcriber. All measurements were transcribed in the order in which they were taken.



<u>Figure 4.</u> An image of OpID 0489 dentition to demonstrate the Universal Numbering System.

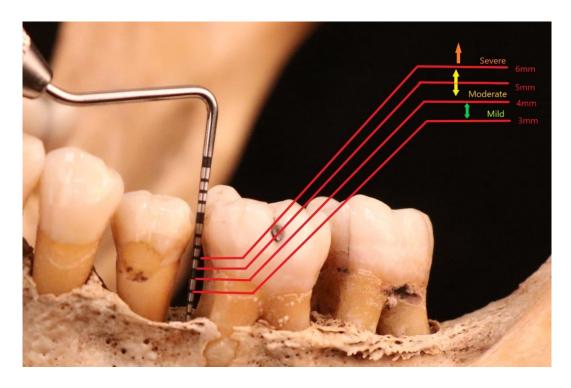
Data Analysis

The raw scores were input into an Excel sheet. The teeth were labeled using the Universal Numbering System. The cells contained the mesial, apical, and distal measurements. The measurements were separated frontal from lingual and maxillary from mandibular for easier assessment. Although third molars were measured, because of the variable presence and the often-angular position, they were excluded from determining severity and presence/absence. One mm was subtracted from the raw scores to account for the average deterioration of the connective tissue space of the connective tissue in order to account for the soft tissue interface known as the biological width (Gargiulo, AW et al., 1961). This adjusts the scores to account for the relative constant of connective tissue attachment, and therefore adjusts the measurements of the skeletal population to reflect that of their living periodontal status. This periodontal severity scoring system, which accounts for the loss of connective tissue attachment, allows skeletal populations' measurements to be objectively compared to living populations (e.g., NHANES) (Gargiulo et al., 1961; Tonetti et al., 2018; Eke et al., 2018).

Conditional formatting was used to identify and color code the severity level of each measurement. If an individual had 2 or more sites with non-adjacent teeth that were 3mm or more, then periodontal disease was noted as present (Eke et al., 2018). After presence or absence was determined, severity was then classified by how great the measurements indicating disease were, according to the periodontal severity scale (Eke et al., 2018) (see Table 4 and Figure 5). An individual's severity score was determined by the greatest severity level with 2 or more non-adjacent measurements (Eke et al., 2018).

<u>Table 4.</u> Clinical severity chart used in Eke et al. (2018).

Clinical Severity Chart				
None	Less than			
2mm				
Mild 3mm				
Moderate 4-5mm				
Severe 6+ mm				



<u>Figure 5.</u> An example of a periodontal probe measurement, how to read the probe, and severity was calculated (prior to the 1mm adjustment)

Notes on presence/absence and severity were put into an Access sheet with the demographic information for all individuals. To avoid bias in rounding, the measurements were taken "blind" by looking at demographic information last. Demographic

information included race/ethnicity, biological sex, age, socioeconomic status (childhood and adulthood), presence or absence of chronic disease and type (CVD, diabetes and hypertension), and the individual's cigarette smoking status. These demographics are risk factors for periodontal disease, and therefore associated with stress. These risk factors were later utilized to make a stress composite score. This score was used to determine the relationship with stress and periodontal severity. Once all the demographic information was obtained the Access sheet was transferred to SPSS statistics 26 for analysis, and coded.

The data was assessed for normality using histograms and Q-Q plots. Due to the non-normal distribution for the number of chronic diseases, nonparametric alternative statistical tests were run to address the relationship between chronic disease and periodontal disease. A Kruskal-Wallis was run to assess if there was a difference in the number of chronic diseases (DV) based on the severity of periodontal disease (IV). I followed up the Kruskal- Wallace tests with a series of Mann-Whitney U tests to identify where these differences occurred. A Mann-Whitney U test was run to assess if there was a significant difference in the number of chronic diseases (DV) based on presence and absence of periodontal disease (IV).

Two correlations were run with the present data. The first correlation assessed the relationship between the number of chronic diseases and periodontal severity. The second correlation assessed a stress composite score and periodontal severity. The stress composite score added one point for every present risk factor. Prior to creating the stress composite score all risk factors received were tested individually for significance by comparing average periodontal severity scores (DV) to individuals with and without

these risk factors (IV). This testing incorporated a series of independent t-tests for dichotomous variables to assess the difference of means, and a one-way ANOVA to assess variation between groups, for multi-leveled risk factors. The purpose of the stress composite correlation, and the risk factor assessment was to assess if severity could provide more accurate and explanatory information than the standard anthropological assessment.

In order to asses the differences in periodontal expression and distribution two populations were compared to one another. The distribution of periodontal severity was tested between the TXSTDSC and Operation Identification samples. A Chi2 test of independence, a statistical test that determines if there is a significant relationship between nominal variables through analysis of distribution, assessed the distribution of periodontal severity between the two populations.

For these analyses, 2-tailed tests were used. The significance level was set to \leq 0.05, meaning a p-value must score of 0.05 or less is considered significant. All p-values \leq 0.10 are considered trending for this research. Trending is when a p-value does not reach the threshold of significance but gravitates toward significance.

3. RESULTS

Descriptive Statistics

There is not an even distribution of periodontal disease within the TXSTDSC. Half of the non-edentulous individuals within the TXSTDSC had moderate periodontal disease (50%), while 27% of the sample had mild periodontal disease. Severe periodontal disease was the least common level of severity within the sample (10.81%), while 12.16% of the TXSTDSC non-edentulous sample did not have periodontal disease according to clinical standards (Table 5).

<u>Table 5.</u> Distribution of periodontal disease severity within the TXSTDSC.

Periodontal	Number of
Severity	Individuals
None	9
Mild	20
Moderate	37
Severe	8
Total	74

Of the 74 individuals sampled, 35 had one or more recorded chronic diseases (47.30%). For the sake of this paper, obvious open-heart surgery (i.e., healed or healing bisected and wired sternum) that was not noted in the medical documentation was not included as CVD or chronic disease. This decision was to prevent an overestimation of chronic disease of CVD. Of the 35 individuals with one or more recorded chronic disease, seven individuals (20%) also had severe periodontal disease. Of the 39 individuals without a chronic disease was present in 16 individuals (45.71%) of the 35 with a reported chronic disease. Out of the 39 individuals without a chronic disease 21

individuals (53.85%) had moderate periodontal disease. Mild periodontal disease was present in ten individuals with a chronic disease (28.57%), and ten individuals without a chronic disease (25.64%). Of the 35 individuals with a reported chronic disease only two individuals (5.71%) did not have periodontal disease. This means that of the 39 individuals without a chronic disease, seven individuals (17.94%) did not have diagnosable periodontal disease.

<u>Table 6.</u> Distribution of periodontal severity across self-identified biological affinity within the TXSTDSC.

Periodontal Severity

Biological	None	Mild	Moderate	Severe
Affinity				
White	9	18	34	8
Hispanic	0	1	1	0
Black	0	1	2	0
Total	9	20	37	8

As stated in previously, a majority of the sample that met the parameters previously set for inclusion were White (Table 6). For this reason, it was decided that there should not be an inclusion of ancestry for comparisons, since the sample size was too small for non-White individuals.

<u>Table 7.</u> Distribution of periodontal disease severity within the OpID sample.

Periodontal Number of

Severity	Individuals
None	19
Mild	17
Moderate	12
Severe	2
Total	50

The OpID sample appeared to be evenly distributed with the exception of individuals with severe periodontal severity (Table 7). Only two individuals (4%) within the OpID sample were diagnosed with severe periodontal disease. Out of the 50 individuals sampled, 12 had moderate periodontal disease (24%). Most of the sample had no periodontal disease (38%), or mild periodontal disease (35%). Since the OpID sample is by definition unidentified, there is no demographic or medical information available to compare. The biological profile is estimated and as a result it was decided that the OpID sample should only be utilized in the "Periodontal Measurement Adjustment" and "Comparison Between Populations" sections below. Therefore, charts and tables included beyond the named sections below are limited to TXSTDSC data only.

Periodontal Measurement Adjustment

The periodontal measurement adjustment affected presence and absence of periodontal disease within both samples. Without that 1mm soft tissue adjustment, both samples would have had only one individual without periodontal disease (98.6% of the

TXSTDSC sample and 98% of the OpID sample). With the 1mm adjustment, there was an 10.9% decrease in periodontal disease for the TXSTDSC sample and a 36% decrease for the OPID.

Q-Q plots and histograms showed that periodontal presence and absence had a normal distribution and were homoscedastic, so a paired t-test was run to compare the presence and absence of periodontal disease before the 1mm correction and after. Presence and absence were dummy coded 0 representing absence, and 1 representing presence. The paired t-test showed that there was a significant difference in periodontal disease without the 1mm adjustment (mean=.9865; SD=.116) and with the 1mm adjustment (mean=.8649; SD=.344), t (73)= 3.179, p=.002. This means that there is a significant difference in presence and absence before the correction and after the correction, with an implication that anthropological studies not using the soft tissue correction likely overestimate periodontal disease.

Periodontal Severity and Chronic Disease

Q-Q plots were run to assess periodontal severity and the amount of chronic diseases present. The Q-Q plots showed that number of chronic diseases did not have a normal distribution. Visual inspection of scatterplots and histograms determined that the number of chronic diseases was not homoscedastic. Since the number of chronic diseases violated both assumptions of normality, non-parametric equations were run to assess the relationship between periodontal severity and chronic disease.

A Kruskull-Wallis Test showed that the number of chronic diseases was not significantly different based on the severity of periodontal disease, $X^2(3) = 7.392$, p=.060.

Although these results are not significant, they are trending, so the lack of significance could be in part because of the small sample size. Since the p-value was trending, a series of follow-up Mann Whitney U tests were run.

A Majority of the Mann Whitney U tests were insignificant. However, individuals with severe periodontal disease (mean rank= 31.00, n=8) had higher amounts of chronic diseases than individuals with no periodontal disease (mean rank= 6.28, n=9), U= 11.5, Z=-2.646, p=.008. Although these results did not have a significant p-value, they had a p-value within the range of trending. Individuals with severe periodontal diseases had a significantly higher number of chronic diseases than individuals with moderate periodontal disease (mean rank= 21.27, n=37), U= 84.00 Z=-2.11 p=.035).

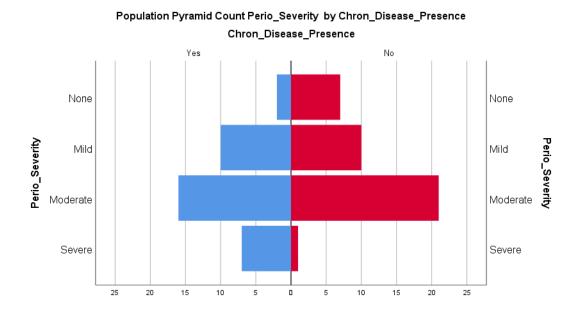
A Spearman's Rho correlation was run to determine the relationship between periodontal severity and amount of chronic diseases present (n=74). The results showed that there was a non-significant positive correlation (r(72)=.211, p=.071, R²= .945). However, the correlation between periodontal severity and chronic disease is trending. The variation in how many of the chronic diseases were present explains approximately 94.5% of the variation in periodontal severity.

Although the number of observed chronic diseases and periodontal severity was not significantly related, all tests provided trending results. This meant that although periodontal disease may not be a successful predictor for the amount of chronic diseases an individual has, there is a clear relationship between both variables.

Periodontal Severity and Stress

A series of t-tests were run to understand the relationship between risk factors and periodontal severity distribution on a univariate level. Assumptions were checked against risk factors and all risk factors had a kurtosis and skewness between -2 and 2. Q-Q plots were visually assessed, and all appeared to be normally distributed. Although there were some outliers for CVD, diabetes, and hypertension, the Q-Q plots appeared to be normally distributed. This means that risk factors met assumptions for homoscedasticity and normal distribution.

An independent t-test was run to assess the difference of means for individuals with chronic disease and without chronic disease based on periodontal severity. Levine's tests show that there is no violation of the variance assumption. There was a significant difference in the means of periodontal severity distribution between individuals who had a chronic disease (n=35; mean=1.80; SD=.833) and individuals who did not have a chronic disease (n=39; mean=1.41; sd=.818), t (72)=-2.028, p=.046. When breaking down the distribution, individuals with chronic disease present have a higher prevalence of severe periodontal disease and a lower prevalence of moderate and no periodontal disease (Figure 6).

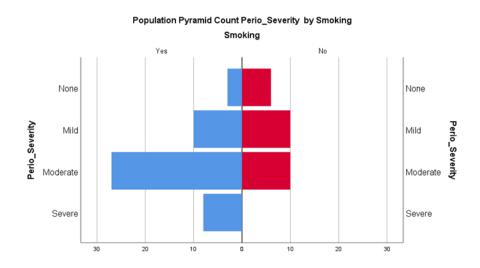


<u>Figure 6.</u> Histogram displaying the distribution of periodontal disease severity based on presence or absence of a chronic disease.

When observing t-tests for presence or absence of specific chronic diseases (hypertension (p=.149), CVD (p=.786), and diabetes (p=.109)) there were no significant results. This means that chronic disease carries a significant difference in distribution of periodontal severity. However, on their own hypertension, CVD and diabetes alone do not have a significant effect on periodontal disease severity.

After determining the significance of chronic diseases an independent t-test was run to assess the difference of periodontal severity between smokers (n=26; =1.833; SD=.781) and nonsmokers (n=48; =1.15; SD=.784). Levene's test for equality of variance did not have a significant result. This means that equality of variance can be assumed. The results show that there is a significant difference between smokers and nonsmokers for periodontal disease severity (t(72)=-3.537; p=.001). Smokers had a higher periodontal severity than nonsmokers. This is shown by the higher prevalence of individuals with moderate and severe periodontal disease in smokers as opposed to the higher prevalence

of no periodontal disease in nonsmoking individuals (Figure 7). This finding reinforces previous findings that smoking is a highly correlated risk factor for periodontitis (Haber, J, 1994; Haber, J et al., 1993).

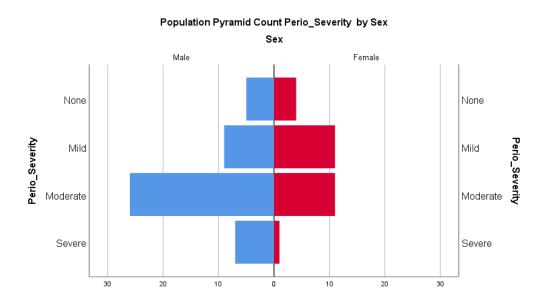


<u>Figure 7.</u> Histogram displaying the distribution of periodontal severity between smokers and nonsmokers.

A 2-way ANOVA was run with childhood and adulthood socioeconomic status (SES) as between-subjects' factors. The results showed that there was no significant effect of childhood SES of adulthood SES on periodontal severity. There is no significant effect of the childhood/adulthood SES interaction and periodontal severity. Although childhood SES is not significant, it has a trending effect on periodontal disease [F (5,54)= 2.207; p= .067]. There is a possibility that childhood SES was affected by individuals with unknown SES. If these unknown SES's were identified it could mean that childhood SES had a significant effect on periodontal severity or that there is no relationship between SES and periodontal severity.

Another independent t-test was run to examine the difference periodontal severity between Males (n=47; mean=1.74; sd= .846) and females (n=27; mean=1.33; sd=.784).

Levene's test for equality of variance did not have a significant result. This means that equality of variance can be assumed. The results show that there is a significant difference between males and females for periodontal disease severity (t(72)=-2.066; p=.042). Males had a higher prevalence of moderate to severe periodontal severity while females had a higher prevalence of mild periodontal disease (Figure 8). This result matched previous literature addressing periodontal distribution between males and females (DeWitte, 2012).



<u>Figure 8.</u> Histogram displaying the distribution of periodontal disease severity based on sex.

The stress composite scores were created to better understand risk factors and potential stress placed on the human body based on demographics. In order to create a composite score, breaking points were chosen for demographics categories. The breaking points consisted of present (+1) and absent (+0) to create a cumulative composite score for each individual (Table 8). SES was excluded from the composite score since the distribution of periodontal disease across different SES categories was even. Although

chronic disease did not have a significant correlation with the number of chronic diseases per individual, it carried some significance, therefore each category was included to create a cumulative result.

<u>Table 8.</u> The breaking points and categories used to create stress composite scores.

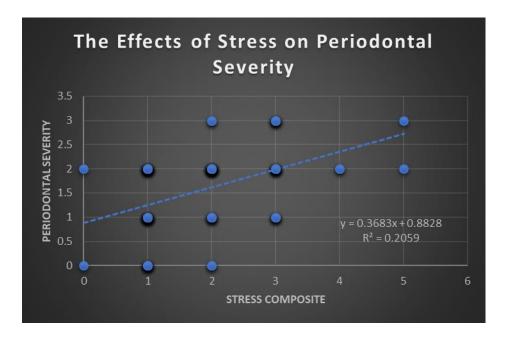
Breaking point

Category	Absent (0pts)	Present (1pt)
Race	White	Minority
Sex	Female	Male
Diabetes	No	Yes
CVD	No	Yes
Hypertension	No	Yes
Other	No	Yes
Smoker	No	Yes

Q-Q plots were run for periodontal severity and stress composite scores. The plots showed that the data were normally distributed, and visual inspection of scatterplots and histograms revealed that the data was homoscedastic. The variable did not violate the assumptions of homoscedasticity and normal distribution, therefore parametric tests were utilized to assess the relationship between periodontal severity and stress composite scores.

A Pearson's correlation was run to determine the relationship between stress and periodontal severity (N=74). Results showed a significant positive correlation r (72)=.454, p<.001, R^2 = .206. The variation in stress composite scores explains approximately 20.6% of variation in periodontal severity. The Pearson's correlation was followed up with a simple linear regression to further explain the effect of stress on periodontal severity. Results showed that stress composite was a significant predictor of periodontal severity, F (1, 72) =18.664, p<.001, R^2 =.206 (Figure 9). For every one

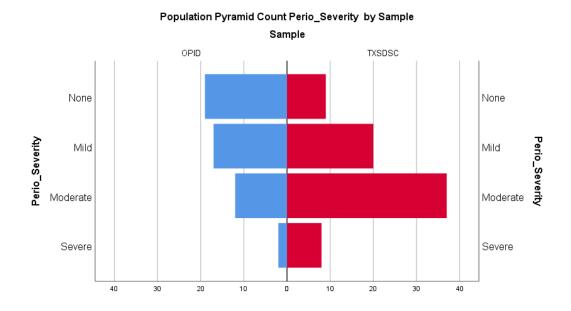
increase to the stress composite score there is a .368 increase in periodontal severity (b= .368, t(72)=4.32, p<.001).



<u>Figure 9.</u> Scatterplot representing the effect of stress on periodontal disease. The stress composite score were used as the independent variable while periodontal severity was dummy coded and used as the dependent variable.

Comparison Between Populations

The last test run was a 2x4 Chi² Test of Independence to examine the relationship between two populations (migrant/TXSTDSC) and periodontal severity (none/ mild/ moderate/ severe) (Figure 10). The relationship between these variables was not significant, $X^2(3, n=124)=16.129$, p=.001. There was is significant difference in frequency of periodontal severities between the two populations (Table 9). This means the migrant population, which has a younger estimated age range of 20-30 years has a significantly different distribution from the TXSTDSC population.



<u>Figure 10.</u> Histogram displaying the distribution of severity split between the OpID sample and the TXSTDSC

<u>Table 9.</u> Frequency distribution of samples by periodontal severity

Sample/Periodont	None		Early/Mild		Moderate		Severe		Total
al Severity									
	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	
OPID	19	11.3	17	14.9	12	19.8	2	4	50
TXSTDSC	9	16.7	20	22.1	37	29.2	8	6	74

4. DISCUSSION

This study aimed to assess the relationship between periodontal disease and severity with chronic disease and stress. In addition, this project sought to incorporate an alternative method of assessing periodontal disease, other than the method regularly used within anthropology, to provide more accurate and explanatory results (e.g., Kerr, NW, 1988; Kerr, NW, 1994). The method was then tested to compare two skeletal samples, one documented and one unidentified, to assess what anthropological interpretations could be made using this alternative method.

The data suggests that a 1 mm adjustment to account for gingival tissue in the living creates a significant increase in individuals without periodontal disease in skeletal individuals. This indicates a probable overestimation of periodontal disease within bioarchaeological studies since they do not account for loss of connective tissue. The analysis confirms that males, smokers, and individuals with chronic disease had a higher average periodontal severity. However, the results show that presence of cardiovascular disease (CVD), hypertension, and diabetes did not have a significant effect on periodontal severity. Similarly, self-identified socioeconomic status (SES) did not exhibit a significant effect on periodontal severity. The study shows that periodontal severity has a significant relationship with stress and an insignificant correlation with the number of chronic diseases present. When comparing the OpID and TXSTDSC samples, the results indicated that there was no significant difference in frequency of periodontal severity between the two populations.

Interpretations

In line with the expectation that there was an overestimation of periodontal disease within anthropological research, this study found that accounting for connective tissue at 1 mm led to a significant reduction in individuals diagnosed with periodontal disease. Taking alveolar measurements but not accounting for loss of soft tissue is one of the issues in anthropological periodontal studies. Additionally, there are anthropological studies that do not use quantitative measurements, but instead visually assess the alveolar bone to take a qualitative approach to diagnosis (e.g., Djuric et al., 2009; Geber and Murphy, 2018; Oyamada, 2010). It may benefit anthropologists to step back from the qualitative macroscopic approach, and instead utilize dental clinical methodology to diagnose periodontal disease within skeletal samples with a quantitative basis that will more accurately reflect the true clinical status of each case.

The expectation positing a relationship between periodontal severity and chronic diseases was that there would be significant relationship. However, the results were complicated. There was no significant difference between individual chronic diseases (CVD, hypertension, diabetes). However, individuals with a chronic disease had a combined higher periodontal severity than those without a chronic disease. While these results may suggest that there is no relationship between periodontitis and chronic disease, considering the numerous papers associating periodontal disease with chronic disease (Belstrøm et al., 2012; Madianos & Koromantzos, 2018; Nazir, 2017) and the distribution of each chronic disease within the TXSTDSC sample, it is likely that a relationship does exist. An alternative explanation is that while the combined chronic

diseases are prevalent within the sample, individually the chronic diseases are underrepresented.

The expectation that periodontal severity had a relationship with the stress composite score was supported. However, the relationship between periodontal disease and chronic diseases was insignificant. This means that periodontal disease cannot be used to estimate presence of a chronic disease, despite the potential existing relationship. On the other hand, periodontal severity is a useful indicator of stress the individual may have endured. This relationship between stress and periodontal severity can be seen through independent risk factors as well as the tested stress composite score (DeWitte, 2012; Geber & Murphy, 2018; Oyamada et al., 2010). The results suggest that for every additional risk factor a there is a .368 increase in periodontal severity. This means that a high severity would indicate an individual with more risk factors, and thus higher stress levels.

The TXSTDSC and OpID sample comparison showed a significant difference in periodontal severity distribution. This shows that periodontal disease can be used to identify significant differences in periodontal severity frequencies based on populations. However, the OpID sample (estimated range of 20-30 years of age) still shows signs of a high prevalence of periodontitis, which indicates an unexpectedly high prevalence of periodontal disease for such a young population. This is supported by Eke et al. (2012), who stated that the chances of getting periodontitis increases with age in a sample drawn from the NHANES studies of United States citizens. Among the individuals examined for the Eke et al. study, 47% of adults above 30 had periodontal disease. The OpID sample (estimated range of 20-30 years of age, excluding outliers) surpasses this percentage

(62%) despite their younger age range. This may show that in this population, periodontal disease may not just be a disease of age, but rather a disease compounded by social and biological stressors (e.g., diet, access to resources, status, political systems, social violence, etc.).

It is important to note that the development of periodontal disease can be related to diet (Nazir, 2017). However, it is unlikely that an individual would develop periodontal disease in adolescence. Periodontal development within children is usually only associated with individuals who are uniquely susceptible to periodontitis (Mros and Berglundh, 2010).

Due to the likelihood that OpID is a migratory population, it is probable that biological, environmental, social, and habitual stressors are factors for the increased periodontal prevalence (Cengiz et al. 2018; Geber and Murphy, 2018; Naorungroj et al., 2017). This includes contributors to stress such as sex of the individual, smoking, and chronic diseases among other risk factors that were not included within this study (DeWitte, 2012; Geber and Murphy, 2018; Naorungroj et al., 2017; Nazir, 2017).

While some OpID individuals might be migrating from Central America seeking to improve their socio-economic levels, this would not account for the high prevalence of periodontal disease. Some would suggest that SES is a cause for this periodontal onset (e.g., Hobdell et al., 2003; Kim et al., 2018), but the data found within this study may suggest otherwise. The results contradict the claims of Hobdell et al. (2003) that SES has a strong relationship with periodontal disease. Within the TXSTDSC sample, self-identified SES was not found to have a significant effect on periodontal severity which backed up findings from other studies (Geber & Murphy, 2018; Oyamada et al., 2010).

Study Limitations

Some of the provided information about the TXSTDSC donors are provided by next of kin. This means that there is the chance that not all donor information is accurate, as next of kin may be missing information regarding an individual's medical status.

Therefore, information on chronic disease is limited, and in some cases, left up to interpretation.

It is beyond the scope of this study to identify if there is a relationship between all chronic diseases and periodontal disease. This study focuses specifically on diabetes, CVD, and hypertension. Conditions that were within the realm of the focused diseases, but unclassifiable, were placed in the "other" category. The exclusion of other diseases could play a role in why the number of chronic diseases were not statistically significantly related, or significantly different based on periodontal severity. This should not be interpreted to mean the inclusion of these diseases would have led to significant results, rather, the inclusion of other diseases could have led to more determining results as opposed to the trending results acquired. Similarly, the inclusion of other diseases could have no effect on the results.

As stated previously, SES provided from the demographics is either self-reported of reported by next of kin. This means that despite SES showing a normal distribution, the use of self-reported SES may be a flawed factor to utilize. Regardless of an individual's income most individuals who self-identify socioeconomic status, report themselves as middle class regardless of childhood family income or adulthood income. This means that the self-reported SES may not be entirely accurate, so it is important to explore other avenues such as occupation to better understand true socioeconomic status.

The TXSTDSC sample had 74 individuals who met the parameters required for this study. However, there were still difficulties in taking measurements. Some individuals had teeth with calculus (hardened plaque), which made reading the CEJ difficult. There were some teeth and alveolar bone that suffered from taphonomic wear, which made teeth and bone brittle and easy to break. Measurements on these spots were not taken to avoid damaging the remains. These are conditions that will be experienced in the field, and the condition of bones in an archaeological context could be in similarly poor condition. For this reason, the restrictions on quality, the number of teeth present, and the number of sites necessary for determining severity are not recommended for bioarcheological studies with poorly preserved teeth and alveolar bone. Instead, one site of periodontal severity can suffice, although two are still preferred, and restrictions on the number of teeth can be reduced.

There is an increased risk of obtaining a Type 1 error due to the use of univariate statistics within this study. Although the use of multivariate statistics could have mitigated the increased chance of obtaining an error, univariate statistics were deemed necessary for this research, because they allowed for a comparison of relationships between each stress factor and periodontal severity.

Future Recommendations

Future studies on periodontal disease should begin considering the subjectivity of visual observations and convert to the use of measurements with a periodontal probe. A measurement is an objective observation that limits biases. Additionally, it is important to account for the loss of soft tissue that occurs during the decomposition process. There is a high chance that periodontal disease has been overestimated simply because the

anthropological protocol for assessing periodontal disease does not regularly account for loss of soft tissue. This overestimation is then amplified by researchers who choose to visually assess their sample exclusively without measurements. If researchers would commit to utilizing periodontal probes for measurement and adjust those measurements by 1mm for all skeletal samples, it would help lessen the overestimation of periodontal disease and allow for more comparative studies.

Since there was a lack of diversity within the TXSTDSC sample, biological affinity and periodontal disease could not be analyzed. Future suggestions from this study would include increasing the modern population to include more minority individuals. This would allow an analysis to assess how much of an impact biological affinity has on the severity of periodontal disease.

Finally, it would be important to follow up this research with another population comparison study. Two populations of the same relative age group, but different environmental conditions would be an ideal secondary comparison. The difference in age groups between the TXSTDSC and the OpID sample is likely a partial cause for the significant results within this project. Although this allowed me to create a more detailed interpretation than originally anticipated, it is important to explore the limits of population comparisons. This will help establish just how important periodontal severity can be in terms of interpreting stress and disease in past populations.

5. CONCLUSION

This research aimed to answer three main questions. The first was to determine if the presence of chronic illnesses had an impact on the presence of periodontal disease.

The second question was if this impact had a correlation with periodontal severity and the number of chronic diseases present in an individual. The third question was to assess if there was a relationship between the composite stress score and periodontal severity.

The results identified that presence of one or more chronic disease had an impact on presence of periodontal disease, but individual chronic diseases did not share this impact. The study also determined periodontal severity had a significant correlation with composite stress scores. However, the correlation between periodontal severity and the number of recorded chronic diseases was indeterminant.

This study also sought to determine the impact of using an adjusted dental clinical method to determine presence and severity of periodontal disease. There was a significant increase in the number of individuals without periodontitis when adjusting for soft tissue. Prior to the adjustment the OpID sample and TXSTDSC had one individual each without periodontal disease, however after the adjustment the TXSTDSC rose to 9 individuals without periodontal disease, and the OpID sample rose to 19 individuals. This means that there is likely an overestimation of periodontal disease in anthropological studies. However, this overestimation did not impact the previously established effects of sex, smoking, or presence of cumulative chronic disease (Dewitt, 2012; Geber and Murphy, 2018; Nazir, 2017). The results contradicted studies that suggest SES and individual chronic diseases might impact periodontal disease, however, more research is

required (Belstrøm et al., 2012; Hobdell et al., 2003; Kim et al. 2018; Madianos & Koromantzos, 2018).

The final portion of this project was to determine the implications of periodontal severity within an undocumented population by comparing it with the TXSTDSC with the use of the adjusted dental clinical approach. With the clinical approach, data suggested that the younger OpID migrant population had periodontal severity frequencies that were significantly different from that of individuals in the older TXSTDSC sample. However, the presence and absence suggested a high level or periodontitis within the OpID sample. Literature suggests that increasing age leads to an increased chance of periodontal disease development, therefore interpretations suggest an increased amount of periodontal disease within the OpID sample, with a significant difference in frequencies of periodontitis. Statistics run for this research suggest that this increase could be related to environmental and social stress but is not significantly related to SES.

The unknown demographics of the OpID sample limits the interpretations that can be made. However, the clinical approach to assessment of periodontal severity allows more insight into population comparison research than macroscopic visual assessments. This research illustrates the need to adopt an objective and quantifiable method, but also raises a question about the relationship between periodontal severity and chronic disease.

Recommendations

Additional exploration for future studies could address the effect of biological affinity/ancestry on periodontal disease, presence, and severity. Literature suggests that ancestry impacts the development of periodontitis, however this relationship has not been

assessed within a skeletal collection (Naorungroj et al., 2017). The effects of ancestry could not be addressed within this project due to the lack of diversity within the TXSTDSC. The impact of ancestry was included in the composite score of this study because of previous literature based on living populations. Therefore, it would be appropriate to explore this effect within a skeletal population and, if necessary, reassess the relationship between periodontal disease and social stress.

Based on these conclusions, anthropological practitioners should consider utilizing the adjusted dental clinical method for assessing periodontal disease, whether it be for presence or severity. This method has two benefits: First, it would decrease the amount of overestimation within the anthropologic record. Second, it would allow for comparisons to occur between skeletal samples, be they archaeological, historic, modern, or even living populations. Third, it would give a more accurate assessment of the true clinical periodontal disease pattern that exists for each case.

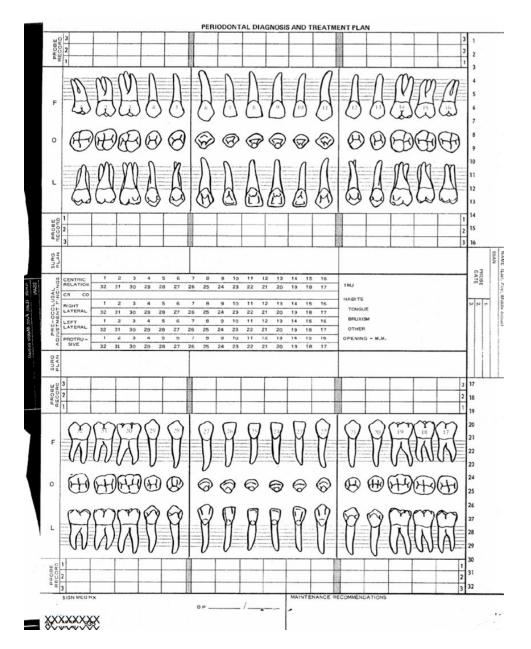
Concluding Remarks

This study has identified and addressed a methodological issue within periodontal disease studies. Not only is the clinical approach not subjective to biases, but it is more accurate, avoids overestimation, and allows for comparisons between living and deceased populations. This can open a variety of new studies within biological anthropology and bioarcheology. Although the effect of chronic disease and periodontal disease appeared to be limited in this project, and there was not a significant correlation between periodontal severity and number of chronic diseases, the relationship with periodontal severity and

stress has been previously established and supported (DeWitt, 2012; Geber and Murphy, 2018; Nazir, 2017; Oyamada et al., 2010).

The knowledge of this relationship combined with the background information of the OpID sample and the demographic information on the TXSTDSC sample allowed for the interpretation that environmental and social stress were possible causes for the rates of periodontal disease within the younger OpID sample. This is just an example of the interpretations that can be made with the use of the clinical method. However, interpretations are only as strong as a researcher's background knowledge. As anthropologists, it is important to have a strong understanding of biological factors that lead to periodontal disease, and the background of our samples prior to making interpretations. In combination with thorough background research, the use of periodontal severity frequencies could add depth to interpretations of skeletal samples.

APPENDIX SECTION



<u>Figure 1</u>. This image is of a periodontal diagnosis chart. This form was used to record the probe measurements for my study. This form is available in the public domain:

Document AF935 available at e-publishing.af.mil.

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