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## Stress, geographic and sociodemographic factors, and oral health outcomes in adolescents and young adults

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A thesis submitted in partial fulfillment of the requirements for the Doctor of Philosophy degree in Medical Biophysics

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## Abstract

Oral health disparities are influenced by socioeconomic and demographic factors. Recent research suggests that stressors can contribute to these disparities. Adolescents and young adults are particularly vulnerable to these stressors, which can affect hypothalamic-pituitary-adrenal (HPA) axis reactivity. This thesis aimed to enhance understanding of the relationships among stress, geographic and sociodemographic factors, and oral health outcomes in adolescents and young adults. Additionally, salivary levels of mRNA encoding the glucocorticoid receptor (GR) were assessed and their relationship to stress and oral health outcomes investigated.

In the first study, the association between neighborhood-level socioeconomic status and dental care outcomes was explored in 2915 patients split into two groups: adolescents (15-17 years) and young adults (18-24 years). Young adults showed significantly worse preventive and treatment outcomes compared to adolescents. Individuals from neighborhoods with lower household income showed a significantly higher cost of dental care, yet worse treatment outcomes.

The second study assessed the association between perceived stress, cortisol levels (hair and saliva), and caries experience in 93 adolescents and young adults without periodontal disease. Results revealed a significant association of dental caries experience with hair cortisol level (a marker of chronic stress) and perceived stress scale score. These associations remained significant even after adjusting for sociodemographic variables.

The third study involved 78 individuals who had also participated in the second study. RNA was isolated from saliva, followed by quantitative real-time polymerase chain reaction to evaluate levels of GR mRNA. mRNA encoding GR $\alpha$  was identified in saliva. Its levels were inversely associated with hair cortisol levels, however there was no significant association with dental caries experience. Thus, additional factors likely contribute to the connection between stress and caries experience. Chronic stress has been associated with reduced expression of GR $\alpha$  and this association appears to hold for GR $\alpha$  mRNA in saliva.

Incorporating individual and community stressors into the analysis of oral health outcomes provides insights into the complex pathophysiological pathways underlying dental disorders. Establishing the association of biomarkers, such as hair cortisol and GR $\alpha$  mRNA levels, with oral disease may encourage further research and open new avenues for focused preventive strategies, diagnostics, and interventions.

## Keywords

Adolescents

Cortisol

Cost of dental care

Dental caries

Dental treatment outcomes

Geographic information system (GIS)

Glucocorticoid receptor

Oral health inequality

Real-time quantitative reverse transcription PCR

Saliva

Saliva pellet

Stress

Young adults

## Summary for Lay Audience

Social and economic position in society is known to influence an individual's oral health. In addition, stress can profoundly impact our mental and physical health, especially during adolescence and young adulthood. We studied whether dental treatments are associated with social and financial status, and the relationships of stress and molecules related to stress with tooth decay in teenagers and young adults.

First, we analyzed how the income of the neighborhood they reside in is associated with dental care in teens (aged 15-17) and young adults (aged 18-24). Young adults seem to experience more challenges in keeping their teeth healthy than do teens. Also, people from poorer neighborhoods paid more for dental care and had worse treatment results.

Second, we checked stress hormone levels, stress scores, and the number of cavities, missing teeth, and filled teeth in teens and young adults. We found that having cavities was linked to feeling stressed and having high levels of the stress hormone cortisol in hair, which reflects long-term stress. This link stayed strong even after considering things like age, income, and parental education.

Third, we looked at participants from our previous research in more detail. We collected their saliva and used a special method called RT-PCR to measure levels of RNA that codes for the protein that functions as a receptor for cortisol. This receptor is known as GR $\alpha$ . We found that RNA for GR $\alpha$  was present in saliva. Moreover, GR $\alpha$  levels were lower in people with higher levels of cortisol in their hair. When people experience chronic stress, they produce more cortisol, which can decrease levels of GR $\alpha$ , as we observed in saliva. In contrast, GR $\alpha$  levels were not associated with whether or not participants had experienced dental cavities.

These findings emphasize the interaction between social and psychosocial factors and the oral health of adolescents and young adults. The results help us understand how mental and social well-being affects oral health and how this may contribute to inequalities in oral health.

## Co-Authorship Statement

This thesis includes three integrated articles that have been or will be submitted for publication. The co-authorship information for each article is shown below.

Chapter 4 was adapted from the publication titled "Neighborhood-Level Inequalities in Dental Care of Adolescents and Young Adults in Southwestern Ontario" published in the journal *Children* in 2022 by Naima Abouseeta, Noha Gomaa, S. Jeffrey Dixon, and Sharat C. Pani. Naima Abouseeta was involved in the study design, data extraction, data analysis, interpretation of data, and manuscript preparation. Drs. Pani, Dixon, and Gomaa participated in the conceptualization and design of the study, interpretation of data, manuscript design, writing, and review. All listed co-authors critically reviewed the work and approved the manuscript submission.

Chapter 5 was adapted from the publication titled " Relationships among Cortisol, Perceived Stress, and Dental Caries Experience in Adolescents and Young Adults" by Naima Abouseeta, Noha Gomaa, Ali Tassi, Abdelbaset A. Elzagallaai, Michael J. Rieder, S. Jeffrey Dixon, and Sharat C. Pani, which is has been accepted for publication by the journal *Caries Research*. Naima Abouseeta was involved in data collection, laboratory experiments, data analysis, interpretation of data, and manuscript preparation. Drs. Pani, Dixon, and Gomaa participated in the conceptualization and design of the study, interpretation of data, manuscript design, writing, and review. Dr. Tassi assisted with the recruitment of patients from the Graduate Orthodontic Clinic and in manuscript review. Drs. Elzagallaai and Rieder provided Naima Abouseeta with training in the protocol for measurement of cortisol, provided access to facilities in the drug safety lab, and assisted with review of the manuscript. All listed co-authors critically reviewed the work and approved the manuscript submission.

Chapter 6 was adapted from the manuscript titled "Profiling mRNA encoding glucocorticoid receptor  $\alpha$  in saliva: Relationship to hair cortisol levels in individuals aged 15-25 years" by Naima Abouseeta, Noha Gomaa, Ali Tassi, S. Jeffrey Dixon, Krishna Singh and Sharat C. Pani, which is has been submitted for publication. Naima Abouseeta was involved in data collection, laboratory experiments, data analysis, interpretation of

data, and manuscript preparation. Dr. Gomaa initially suggested the concept of the study. As well, Drs. Pani, Singh, Dixon, and Gomaa participated in the conceptualization and design of the study, interpretation of data, manuscript design, writing, and review. Dr. Singh provided Naima Abouseta with training in the protocols for RNA extraction and quantitative reverse transcription PCR. As well, he provided access to facilities in his laboratory. Dr. Tassi assisted with the recruitment of patients from the Graduate Orthodontic Clinic and in manuscript review. All listed co-authors critically reviewed the work and approved the manuscript for submission.

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## List of Abbreviations, Symbols and Nomenclature

ACTH	Adrenocorticotropic Hormone
ANOVA	Analysis of Variance
ANS	Autonomic Nervous System
B	Unstandardized Coefficient
Beta	Standardized Coefficient
CAD	Canadian Dollar
CDCP	Canadian Dental Care Plan
cDNA	Complementary DNA
CHMS	Canadian Health Measures Survey
CI	Confidence Interval
CNS	Central Nervous System
CPI	Community Periodontal Index
CT	Cycle Threshold
CVD	Cardiovascular Disease
DBD	DNA-Binding Domain
DEPC	Diethyl Pyrocarbonate
DMFT	Decayed, Missing, and Filled Teeth
DNA	Deoxyribonucleic Acid
DQA	Dental Quality Alliance

ECC	Early Childhood Caries
ELISA	Enzyme-Linked Immunosorbent Assay
Esri	Environmental Systems Research Institute
F	Fischer's ANOVA Coefficient
FSA	Forward Sortation Area
GAPDH	Glyceraldehyde 3-Phosphate Dehydrogenase
GAPDH-F	GAPDH Forward Primer
GAPDH-R	GAPDH Reverse Primer
GBD	Global Burden of disease
GIS	Geographic Information System
GR	Glucocorticoid Receptor
GR $\alpha$ -F	Glucocorticoid Receptor $\alpha$ Forward Primer
GR $\alpha$ -R	Glucocorticoid Receptor $\alpha$ Reverse Primer
GR $\beta$ -F	Glucocorticoid Receptor $\beta$ Forward Primer
GR $\beta$ -R	Glucocorticoid Receptor $\beta$ Reverse Primer
GRE	Glucocorticoid response element
GS	Glucocorticoids
HPA	Hypothalamic-Pituitary-Adrenal
HSO	Healthy Smiles Ontario

HSREB	Health Sciences Research Ethics Board
LBD	Ligand Binding Domain
LC-MS/MS	Liquid Chromatography–Tandem Mass Spectrometry
LSD	Least Significant Difference
MR	Mineralocorticoid Receptor
mRNA	Messenger RNA
N	Population Size
n	Sample Size
NR3C1	Nuclear Receptor Subfamily 3 Group C Member 1
NTD	N-Terminal Domain
ODA	Ontario Dental Association
OEV-CH-A	Routine Oral Evaluation
OLS	Ordinary Least Squares
OR	Odds Ratio
p	Probability Value
PCR	Polymerase Chain Reaction
PRV-CH-A	Preventive Services
PSS	Perceived Stress Scale
qPCR	Quantitative Real-time PCR
r	Pearson Correlation Coefficient

RCF	Relative Centrifugal Force
Rho ( $\rho$ )	Spearman's Correlation Coefficient
RNA	Ribonucleic Acid
RT	Room Temperature
RT-PCR	Reverse Transcription-Polymerase Chain Reaction
SES	Socioeconomic Status
Sig	Level of Statistical Significance
SW	Shapiro Wilk
t	t Coefficient
TRT-CH-A	Dental Treatment Services
USD	United States Dollar
WHO	World Health Organization

## Chapter 1

### 1 Introduction and Thesis Overview

Even though most oral illnesses are treatable, they remain global public health issues. Overall, oral diseases lead to severe health and economic burdens, and can significantly reduce the quality of life for those affected [Watt et al., 2016; Peres et al., 2019]. The most widespread and significant oral diseases on a global scale include periodontal disease and dental caries, posing significant challenges to public health [Petersen et al., 2005]. Oral diseases are generally characterized by their chronic and advancing nature. For example, dental caries can first appear in very young children and extend throughout adolescence and adulthood into the later stages of life.

Although oral diseases can affect individuals from all socioeconomic backgrounds, several studies have shown that socioeconomically disadvantaged groups are particularly impacted by the financial burden of oral diseases [Farmer et al., 2016; Koga et al., 2020; Zivkovic et al., 2020; Fang et al., 2021]. These groups include individuals with low family income, low levels of education, and those residing in certain geographic areas. These populations also tend to experience greater barriers to accessing dental care and are at a higher risk of poor dental health outcomes than more privileged populations [Strömberg et al., 2012; Zivkovic et al., 2020].

Similar to disparities observed in other chronic diseases, socioeconomic inequalities in oral health extend beyond a simplistic dichotomy between the rich and the poor. Instead, they are characterized by a socioeconomic gradient, where oral health declines progressively throughout the social stratification from top to bottom. This gradient remains consistently evident across various indicators of socioeconomic position, and has been shown for dental caries, periodontal diseases, and tooth loss [Elani et al., 2012; Farmer et al., 2016].

The mechanisms driving socioeconomic inequalities in oral health still need to be understood more adequately. Our lack of understanding continues to impede progress in addressing these inequalities. In contrast, there is an extensive body of literature on socioeconomic disparities in overall health [Krieger, 2005; Solís, 2018]. Proposed theories

encompass material, behavioral, and psychosocial explanations for these disparities [Sisson, 2007; Duijster et al., 2018]. Although several studies highlight the role of socioeconomic and behavioral factors in oral diseases, there is relatively limited research exploring the impact of stress-induced physiological dysregulation on oral health outcomes such as dental caries [Hertzman and Boyce, 2010; Tikhonova et al., 2018]. Moreover, how stress can contribute to oral diseases has not been extensively investigated [Vasiliou et al., 2016; Tikhonova et al., 2018]. Furthermore, only a limited number of studies have examined the association of biological markers with social inequalities in oral health.

Psychological stress can lead to brain alterations and physiological disruptions that impact health and developmental outcomes across the lifespan. This exposure can be particularly harmful during adolescence and young adulthood due to significant changes in HPA axis reactivity [Siqueira et al., 2000; Lenz, 2001; Findlay, 2017]. Investigation of biological markers is one approach that may help explain the pathways linking exposure to chronic stress to oral health outcomes. Cortisol is the most widely studied marker of the HPA axis activity. Cortisol levels in hair and saliva been shown to be a reliable marker of stress, but their association with dental caries is not well understood [Russell et al., 2012; Tikhonova et al., 2018]. Recent evidence from our epidemiological studies suggests that sociodemographic variables, particularly income level, may contribute to social stress, ultimately influencing oral health outcomes [Podskalniy et al., 2021]. However, it is unclear if individual variations in the expression of stress markers are associated with social challenges and oral health outcomes and social challenges. In the last decade, there have been efforts to investigate the expression of the glucocorticoid receptor as a measure of activation of the HPA axis. Recent evidence indicates that glucocorticoid receptor expression could serve as a reliable measure of its activation [Vassiliou et al., 2019; Panagiotou et al., 2021], and would be free of the potential confounding influence of oral inflammation on cortisol levels.

This dissertation utilized existing billing data and recruited patients from the dental clinics of the Schulich School of Medicine & Dentistry. One goal was to investigate the patterns of dental treatment utilization in adolescents and young adults and examine the impact of sociodemographic variables on these outcomes. The project then addressed the interplay



among the biological, social, geographical, and living conditions that affect oral diseases and associated inequalities. Some of the research questions that this study addressed were as follows: Are there differences in dental treatment utilization as individuals transition from adolescence to adulthood? Are socioeconomic factors and stress levels associated with dental caries in adolescents and young adults? To what degree is the stress response associated with oral health outcomes?

This thesis comprises three studies. The first investigated whether the association of neighborhood-level socioeconomic status with dental care costs and outcomes differs between adolescents and young adults (Chapter 4). The second was a clinical study that utilized primary data from a population-based sample to assess the relationships among stress, cortisol levels (in hair and saliva) and the overall caries experience of adolescents and young adults (Chapter 5). In the third study, we measured salivary levels of mRNA encoding the glucocorticoid receptor to investigate the relationships among stress, and socioeconomic factors, and dental caries experience of adolescents and young adults (Chapter 6).

This dissertation demonstrates that adverse socioeconomic exposures at individual and community levels are linked to dental caries experience. It indicates the association of psychosocial factors and oral health outcomes. Hair cortisol levels were found to be inversely related to salivary levels of mRNA encoding the glucocorticoid receptor. These findings suggest potential paths for future investigation of stress and sociodemographic factors within the context of oral diseases.

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## Chapter 2

### 2 Literature Review

#### 2.1 Chapter Summary

This dissertation examines socioeconomic disparities in oral health among adolescents and young adults and attempts to understand the role of psychosocial factors. Adolescence is the developmental stage between childhood and adulthood, typically from 10 to 19 years. Young adults usually refer to people in their late teens and early twenties or those in their twenties, from 19 to 25 years [Birch, 1997]. This literature review is organized into four parts. It begins with an overview of oral health in adolescents and young adults. It then describes the central concepts used in this dissertation, including dental caries, gingival health and disease, and trends in oral health and disease among adolescents and young adults. Also, I review our understanding of the complex interplay between social and biological factors in oral disease. Next, I discuss the concept of oral health inequalities and examine the role of geographic and economic inequalities, immigration, and marginalization in contributing to oral health disparities among adolescents and young adults. The chapter also explores theories explaining these inequalities, including the biomedical model and biopsychosocial model, and the influence of socioeconomic status, health behavior, and biological factors on oral health outcomes. Finally, I review the HPA axis and its relevance to oral health. Regulation of the HPA axis and the potential dysregulation that can occur in relation to stress are examined. I also discuss cortisol, a stress hormone, and its association with dental caries and glucocorticoid receptors, which influence the body's response to stress, and may have implications for oral health.

#### 2.2 Oral Disease in Adolescents and Young Adults

Oral diseases, a substantial public health concern, include dental caries, periodontal diseases, infections, and cancers [Peres et al., 2019]. Worldwide, approximately 3.5 billion individuals are impacted by primary oral diseases and conditions – a global prevalence of 45%, surpassing that of all other non-communicable diseases [WHO, 2022]. Dental caries and periodontal diseases are the most widespread oral pathologies, impacting quality of

life and affecting 35% and 10% of the global population, respectively [Kassebaum et al., 2015].

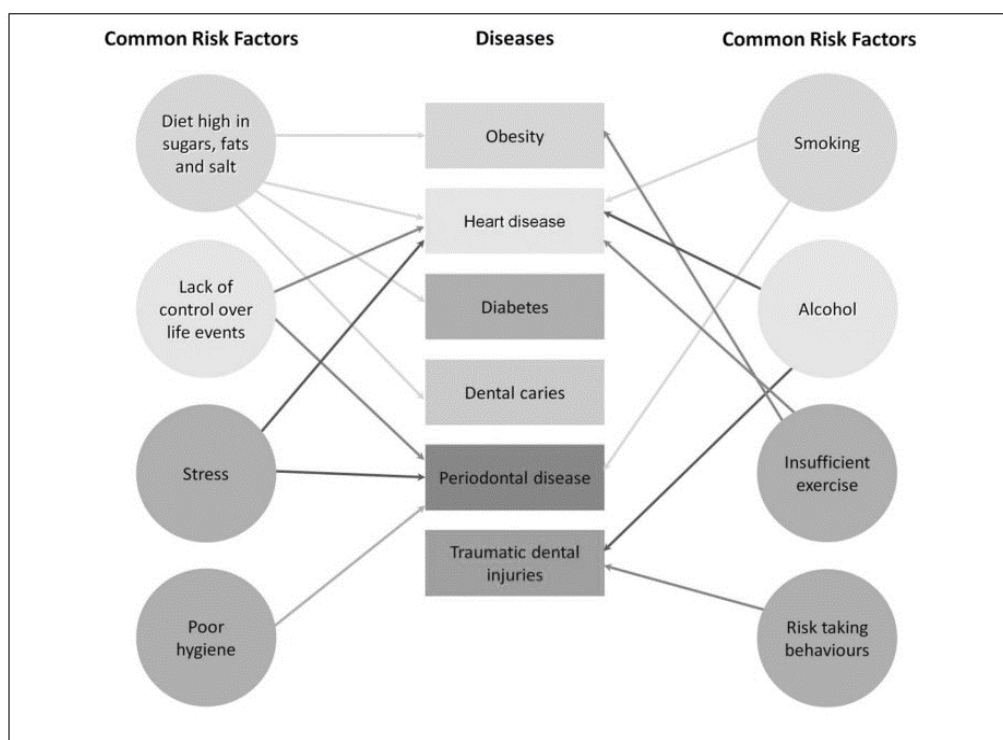
Oral diseases significantly impact the health and well-being of adolescents and young adults. *Adolescence* is a critical developmental phase bridging childhood and adulthood. It is characterized by biological, psychological, and social changes that greatly influence health-related behaviors [Lenz, 2001; American Academy of Pediatric Dentistry, 2018]. During adolescence, crucial oral health behaviors like self-care, diet, and regular dental check-ups take root and often continue into adulthood. As adolescents become more socially independent, they may face hurdles in obtaining proper oral health education and maintaining regular dental visits [American Academy of Pediatric Dentistry, 2018; Jessani et al., 2021]. Research indicates that inadequate oral hygiene habits during childhood and adolescence tend to persist into adulthood [Sheiham and Watt, 2000; Peres et al., 2011]. Additionally, it has been showed that experiencing dental caries during childhood is linked to an increased risk of tooth decay in adulthood [Manton, 2018], malocclusions, and oral infections [Peres et al., 2019]. It has been shown that, as children transition into adolescence and adulthood, sociodemographic factors and oral health-related behaviors are associated with levels of gingivitis and periodontitis [Pitts et al., 2011; Schroth et al., 2013; Fan et al., 2021].

Adolescence can be a critical phase for periodontal health. Evidence suggests that irreversible tissue damage associated with periodontal diseases often starts in late adolescence and early adulthood. During this period, adolescents are likely to exhibit a higher prevalence of gingivitis than prepubertal children or adults [Jenkins and Papapanou, 2001; Cole et al., 2014].

Thus, addressing oral health in adolescents and young adults is a significant public health priority. However, it has been noted that research and preventive programs for oral health have frequently overlooked the specific needs of young adults between the ages of 18 and 25 [Roberts-Thomson and Stewart, 2008].

## 2.2.1 Dental Caries

Dental caries is the most common chronic disease [Pitts et al., 2017]. It is a multifactorial disease that involves interactions among the tooth surface, bacterial biofilm, dietary sugars, and host factors [Mouradian, 2001; Pitts et al., 2017]. Dental caries share numerous risk factors with various other chronic non-communicable diseases (Figure. 2.1) [Sheiham and Watt, 2000]. Modifiable risk factors for chronic diseases often involve lifestyle choices, such as tobacco and alcohol use, diabetes, obesity, dietary factors, and stress.



**Figure 2.1: Common risk factors for chronic and oral diseases [Sheiham and Watt, 2000]. Used with permission of John Wiley & Sons - Books, from “The common risk factor approach: a rational basis for promoting oral health”, Sheiham, Aubrey; Watt, Richard Geddie, 28, 399-406, 04-01-2000; permission conveyed through Copyright Clearance Center, Inc.**

Caries prevalence has decreased markedly in Canada over the past 40 years. According to the Canadian Health Measures Survey [CHMS. Health Canada, 2010], the percentage of children (6-11 years of age) with at least one decayed tooth decreased from 74% in the

1970s to 24% in the 2000s. Similarly, the average number of decayed, missing and filled teeth decreased from 17.5 to 10.7, and the percentage of adults with no natural teeth decreased from 24% to 6% (Figure. 2.2). Additionally, the results from the CHMS, 2010 demonstrate that the percentage of adolescents (2-19-year-olds) with at least one decayed tooth decreased from 96.6% to 58.8%. Among Canadian adults (20–79 years of age), 95.9% have experienced coronal caries, while 20.3% have experienced root caries, 30% of which remain untreated. Improved clinical dentistry, use of fluorides, and public health programs have led to this decrease in the occurrence of oral diseases.

Despite recent advances, people do not have equal access to and benefit from these services [Schroth et al., 2005; Pierce et al., 2019]. Certain groups, like First Nations, Inuit, new immigrants, low-income households, and those in remote areas, continue to experience high rates of dental caries [Fang et al., 2021; Park et al., 2021]. This demonstrates the importance of analyzing risk factors as well as preventive and dental care interventions.

Indicator	Canada Nutrition Survey 1972	Canadian Health Measures Survey 2009
% of children (6–11 years of age) who had at least one cavity (DMFT)	74% (Ages 8–10)	< 25%
Average number of Decayed, Missing, Filled Teeth (DMFT) on children 6–11 years of age	2.5 (Ages 8–10)	0.49
% of adults (who have teeth) who had at least one cavity	96%	96%
Average number of Decayed Missing Filled Teeth (DMFT) in adults who have teeth	17.5	10.7
% of adults who have lost their teeth	24%	6%

**Figure 2.2: Comparison of the National Canadian Oral Health Clinical Survey (1972) to the Canadian Health Measures Survey 2007–2009 [CHMS. Health Canada, 2010]. © All rights Health Measures Survey 2007–2009. Health Canada. Reproduced with permission from the Minister of Health, Canada, 2023.**

Several studies have been published that establish an association between oral health and socioeconomic status [Watt and Sheiham, 2012]. Recently, concern has shifted toward understanding environmental factors (housing and working conditions), psychosocial factors (stress, social support, and behavioral issues), and biological factors that might influence dental caries [Silva et al., 2018].

Psychological stress and adverse social conditions often trigger behaviors like smoking, excessive alcohol consumption, and overeating among individuals, all of which can impact oral health [Sabbah et al., 2018]. It has also become clear that the social environment impacts oral health, depending on how individuals of varying socioeconomic backgrounds interact with their economic and social contexts [Watt and Sheiham, 2012].

Boyce and coworkers reported a significant association among low socioeconomic position, elevated basal activity of children's HPA axis, higher numbers of cariogenic bacteria, and increased risk of dental caries [Boyce et al., 2010]. This led Boyce and coauthors to suggest that stress contributes to an elevated presence of cariogenic bacteria and, consequently, increased susceptibility to dental caries.

Despite these insights from previous studies, significant aspects still require empirical evaluation. One such aspect is insufficient research on oral health and its correlation with social and psychosocial factors contributing to oral disease disparities among adolescents and young adults aged 15-25 years. Thus, investigating oral disease disparities in relation to stress processes may provide better understanding of the complex mechanisms that link socioeconomic position and oral health in adolescents and young adults.

### 2.2.2 Gingival Health and Disease

Gingivitis and periodontitis are inflammatory conditions that affect the tissues that support teeth. Gingivitis affects only the gingiva, whereas periodontitis affects both the soft and hard tissues of the periodontium.

Adolescents experience varying degrees of gingivitis, while periodontitis can affect permanent teeth across all age groups [Oh et al., 2002]. During puberty, gingival tissue becomes more susceptible to irritants, such as plaque, calculus, and food debris, due to a



rise in sex hormones [Chaitra et al., 2012]. Thus, gingival inflammation may be increased during puberty [Jenkins and Papapanou, 2001]. There are several additional factors that affect the risk of gingivitis, including oral hygiene, other oral health behaviors, gender, the presence of dental calculus, and socioeconomic factors [Kassab and Cohen, 2003]. Adolescents from a low socioeconomic background are more prone to developing gingivitis and experiencing more cavities than those from a high socioeconomic background [Gazzaz et al., 2021]. According to the Canadian Health Survey conducted between 2007 and 2009, approximately 32% of Canadian adults between the ages of 20 and 79 have gingivitis [CHMS. Health Canada, 2010]. Identifying and treating gingivitis in young individuals is crucial because continued gingivitis can lead to the development of periodontitis [Lang et al., 2009]. Periodontal diseases are linked to plaque accumulation and the host's immune response. The severity and progression of periodontal diseases are influenced by systemic, genetic, social, psychological, and socioeconomic (income and education) variations, and behavioral factors such as smoking [Gomaa et al., 2020].

There is a social gradient in periodontal disease, in which the disease is more prevalent and severe at the bottom of the social scale and minimal at the top. Adverse social conditions and lower social standing are associated with engaging in health-risk behaviors, while higher social status is linked to better health behaviors [Gomaa et al., 2020; Gazzaz et al., 2021]. Several studies have explored the association between stress and periodontal disease. These studies have revealed that chronic stress and inadequate coping mechanisms are potential risk factors that contribute to the onset of periodontitis [Obulareddy et al., 2018; Castro et al., 2020]. The underlying mechanism may involve stress weakening the immune system and thereby increasing the risk of periodontal disease. Stress can also lead to unhealthy behaviors, like smoking and neglecting oral hygiene [Irwin et al., 1990; Murakami et al., 2018; Sabbah et al., 2018; Gomaa et al., 2020]. Additionally, stress can directly impact periodontal health by altering salivary composition, disturbing the gingival blood circulation, and affecting the body immune response by releasing of stress hormones, such as cortisol [Khodaii et al., 2019].

Cortisol levels are commonly used as biomarkers of stress [Hellhammer et al., 2009]. However, periodontal inflammation can lead to elevated cortisol levels due to the body's

response to inflammation [Breivik et al., 1996; Weik et al., 2008]; therefore, cortisol levels may not be solely indicative of psychological stress. Researchers conducting stress-related studies using cortisol measurements need to be aware of the potential confounding effect. To minimize this confounding factor, researchers should assess the oral health status of participants and consider excluding from their studies individuals with gingival inflammation or periodontitis. In this way, researchers would eliminate the potential influence of periodontal inflammation on cortisol levels. This issue has been considered in our study.

Overall, the findings reviewed above emphasize the need to consider social determinants, such as socioeconomic status and psychosocial factors, when aiming to improve oral health outcomes in adolescents and young adults.

### 2.2.3 Global Trends in Oral Health and Disease

The aim of the World Health Organization (WHO) “World Oral Health Program” is to advance and sustain good oral health in communities by emphasizing preventive measures over restorative dentistry [Elderton, 2003]. In their recent statement in 2021, the WHO released a draft global strategy on tackling oral diseases by 2023. Goals were to identify effective oral health interventions and translate them into an action plan to reduce global oral health issues [WHO, 2022]. The WHO emphasized the importance of addressing disease reduction and the underlying causes of disease. These require a systematic approach to tackle social, environmental, and economic determinants of health, many of which extend beyond the current scope of the healthcare sector.

Data from the “Global Burden of Disease Collaborative Network Global burden of disease study” in 2019 showed that approximately 3.5 billion people have oral health issues [GBD, 2023]. These conditions include the presence of untreated dental caries in primary and permanent dentitions, progressive periodontal disease, and tooth loss [Peres et al., 2019]. Periodontal disease affects 20% to 50% of people worldwide [Sanz et al., 2010], and there was a 57% increase in its burden from 1990 to 2010 [Nazir et al., 2020].

Permanent teeth with untreated caries emerged as the most widespread oral health disorder in 2010 [Kassebaum et al., 2015], affecting approximately 35% of the global population (approximately 2.4 billion people). These data were obtained from an extensive study involving 186 research investigations covering individuals aged five or older across 67 countries [Kassebaum et al., 2015]. Additional study found that untreated caries in permanent teeth continue to be the most common oral disease, with a prevalence of 34% [Kassebaum et al., 2017]. The highest prevalence was observed in younger individuals, specifically among 15 to 19-year-olds.

Global trends in oral disease show a clear impact of social gradients [Sanders et al., 2009; Gazzaz et al., 2021]. A global survey conducted between 2017 and 2018, covering 101 countries classified by the World Bank Atlas Method, revealed that most high-income countries provided emergency dental care for children and adolescents, while this service was less common in low-income countries [Petersen et al., 2020]. Middle-income countries had a particularly high rate of dental caries experience among adolescents, while high-income countries showed relatively high levels of dental caries among young and older adults [Petersen et al., 2020].

These trends are evident across various socioeconomic indicators, including income, education, and social class [Koga et al., 2020]. Disadvantaged members of society, particularly those with lower income and education levels, tend to suffer from oral diseases more frequently compared to their more affluent counterparts [da Cunha et al., 2017; Petersen et al., 2020]. The unequal distribution of oral disease is noticeable not only in low and middle-income countries (Bhandari et al., 2016), but also in countries with high income [Chari et al., 2022]. In high-income countries, linear trends have been observed, with individuals from lower income groups being more susceptible to disease as compared to their more well-to-do counterparts [Farmer et al., 2016].

These findings emphasize the effect of socioeconomic factors on oral health outcomes. Also, the persistent global burden of untreated dental caries in permanent teeth needs to be addressed with continued efforts in preventive care, particularly among young individuals.

Addressing disparities requires targeted interventions and policies to promote equitable access to dental care and improve oral health outcomes for all segments of the population.

#### 2.2.4 Understanding Socio-Biological Interactions in Oral Disease

The field of social-biological interactions in oral disease is continuously evolving and complex. It aims to reveal how social factors influence biological processes and lead to discrepancies in oral health among different public groups. While there is substantial evidence of social factors influencing oral disease development, the underlying biological pathways are poorly understood [Boyce et al., 2010]. Consequently, interdisciplinary approaches integrating social and biological sciences have gained prominence in the study of oral diseases and their causes [Gomaa et al., 2016] .

Traditional approaches to preventing and managing dental diseases have focused on biomedical and behavioral models. For instance, dental caries is primarily influenced by factors related to diet and the host, whereas periodontal disease results from the interaction between microbial plaque and the host's immune response. However, solely targeting these factors may prove less effective in preventing dental diseases, as current interventions often concentrate on eliminating one cause, such as *Streptococcus mutans* in prevention of dental caries.

Empirical evidence suggests that both behavioral and biomedical interventions may not effectively reduce health disparities and may even exacerbate social inequalities in health. This may be attributed to a lack of understanding regarding the interplay between social determinants and biological responses and their impact on common risk factors associated with oral diseases [Gomaa et al., 2016] . Further discussion of oral health inequalities can be found in section 2.3 below.

To gain a more comprehensive understanding of oral diseases, researchers have explored a biopsychosocial model that combines biology, psychology, and social factors. Chronic psychosocial stress resulting from social inequality, such as low socioeconomic status and unfavorable interpersonal relationships, has been linked to alterations in physiological systems, including the HPA axis and the immune response [Baiju et al., 2017; Gomaa et

al., 2019; Ao et al., 2020]. These alterations can significantly affect disease-related behaviors and oral diseases, as well as exacerbate other diseases, such as cardiovascular disease, obesity, asthma, and depression [Marmot et al., 1991; Kiecolt-Glaser and Glaser, 2002; Steptoe et al., 2002; Gleit et al., 2007; Quinn et al., 2010; Ippoliti et al., 2013].

The concept of allostatic load refers to the gradual damage inflicted by chronic stress on body systems, including the immune, metabolic, cardiovascular, and respiratory systems, rendering individuals vulnerable to chronic diseases [McEwen and Stellar, 1993]. Disruptions in the HPA axis and the sympathetic-adrenal-medullary system can release stress hormones, including cortisol, epinephrine, and norepinephrine. In turn, these hormones can adversely affect physiological systems, including the neuroendocrine, immune, and metabolic systems [McEwen, 2013]. Additionally, lifestyle choices and behaviors such as smoking, diet, exercise, and alcohol consumption can influence the interaction of physiological systems with stress [Acharya and Pentapati, 2012].

Low socioeconomic status, alteration of children's HPA axis, and increased counts of cariogenic bacteria independently increase the chance of dental caries in children [Boyce et al., 2010]. Further research indicates alterations in saliva composition, characteristics, and microbial adherence following exposure to stressful conditions, suggesting a potential correlation between stress and oral health [Bosch et al., 2003; Tikhonova et al., 2018]. In adults, chronic psychological stress has been linked to reduced levels of stimulated saliva flow [Hugo et al., 2008].

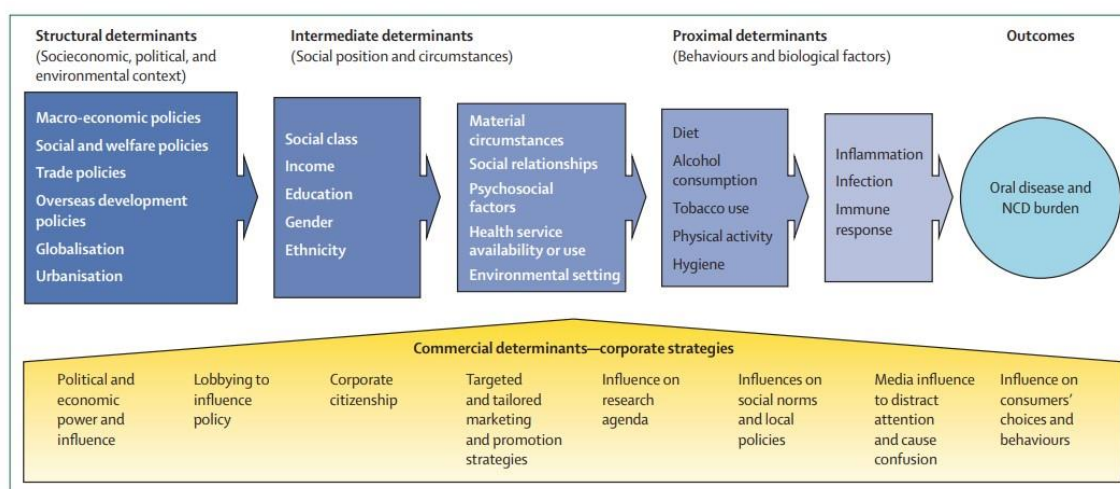
Understanding social-biological interactions in oral disease is essential for comprehending the root causes of oral health disparities. By exploring how social determinants affect biological processes, researchers can gain valuable insights into the complexities of oral diseases. Such insights may lead to the development of preventive approaches and interventions that address both social and biological factors.

## 2.3 Oral Health Inequalities

Health inequalities involve variations in disease prevalence, health consequences, and access to healthcare among different segments of the population. Extensive research has

demonstrated that factors such as socioeconomic status, race/culture, gender, language, and geographical location contribute to these disparities [Carter-Pokras and Baquet, 2016; Watt et al., 2018]. This dissertation examines geographic location, socioeconomic status, immigration, and marginalization factors when examining oral health inequalities.

To enhance oral health and diminish these disparities, it is imperative to address the fundamental causes and gain a comprehensive understanding of the risk factors and socioeconomic, commercial, behavioral, and biological determinants involved, as illustrated in Figure. 2.3 [Peres et al., 2019].



**Figure 2.3: Social and commercial determinants of oral diseases [Peres et al., 2019].** Used with permission of Elsevier Science & Technology Journals, from “Oral diseases: a global public health challenge”, Peres, Marco A; Macpherson, Lorna M D; Weyant, Robert J; Daly, Blánaid; Venturelli, Renato; Mathur, Manu R; Listl, Stefan; Celeste, Roger Keller; Guarnizo-Herreño, Carol C; Kearns, Cristin; Benzian, Habib; Allison, Paul; Watt, Richard G, 394, 249–60, 2019; permission conveyed through Copyright Clearance Center, Inc.

### 2.3.1 Geographical Inequalities

Geographic inequalities in oral health pertain to differences in oral health outcomes and dental care accessibility across different regions. These disparities are influenced by several social and environmental factors, along with the socioeconomic status of the

population [Pitts et al., 2011; Watt, 2012]. Access to oral healthcare is affected by the proximity of dental facilities to patients' residences. Several factors influence how far an individual is willing to travel for dental care services, including the kind of treatment required, the cost of dental care services, travel time and expenses, and the level of economic deprivation in their region [Veginadu et al., 2023].

In Canada, as of January 2016, there was a population-to-dentist ratio of 1,622 individuals for every dentist. However, this ratio varies significantly across provinces, with notable disparities in the availability of dentists between urban and rural areas, especially in remote regions [Canadian Dental Association, 2017]. Rural and remote areas often exhibit geographic disparities in oral health for several reasons, including limited access to oral healthcare, lower proportion of dentists compared to urban areas, absence of community water fluoridation, and less insurance coverage [Ogunbodede et al., 2015; Badri et al., 2021].

Recognizing the significance of geographical locations as indicators of oral health inequalities, Canada's healthcare system employs geo-mapping to identify oral health risks based on residential locations. Geo-mapping is a powerful technique that allows for the visualization and analysis of patterns and distribution of oral health disorders within a geographical area [Schroth et al., 2015a].

Statistics Canada maintains detailed demographic records for the population according to their associated forward sortation area (FSA) code for residential addresses, represented by the first three characters of Canadian postal codes [Schroth et al., 2015a]. FSA codes offer a detailed geographic breakdown, facilitating thorough examination of population health characteristics, including oral health outcomes and disparities [Podskalniy et al., 2021]. Although detailed sociodemographic data is available by FSA code, its correlation with dental caries prevalence in Canada still needs to be explored. Our study used geographic information systems (GIS) models to create maps that predict how geography influences oral health outcomes. This approach can help identify communities in greatest need of oral health support and services, ultimately reducing geographic inequalities in access to dental care.

### 2.3.2 Economic Inequalities

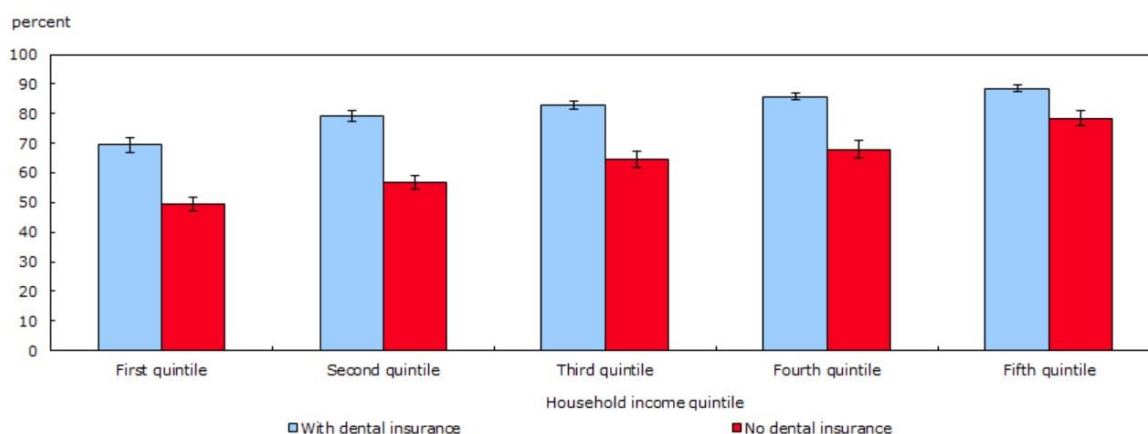
Income, a critical determinant of health, strongly reflects living standards in health research [Bastani et al., 2021]. Globally, research indicates that income inequality within a nation significantly impacts the use of dental care services [Bhandari et al., 2015]. Previous studies consistently link income to disparities across various oral health outcomes [Sabbah et al., 2009a; Singh et al., 2019].

In the Canadian context, the economic landscape is crucial in driving oral health disparities. Access to dental care is tightly bound to affordability, influenced by service costs and disposable income [Fang et al., 2021]. Socioeconomic factors, including income distribution and employment status, play a pivotal role in determining access to quality oral healthcare. While Canada historically maintained relatively higher income equality during the 1970s, subsequent years have witnessed an increase in income inequality [Farmer et al., 2016]. This shift has created financial barriers to oral healthcare, particularly impacting those with lower incomes.

Until recently, universal health insurance in Canada did not cover oral healthcare, which left only 5.5% of the population with public dental insurance, which primarily target low-income individuals [Fang et al., 2021]. Most Canadians' dental treatment expenses were covered through private insurance or personal funds. Adolescents and youth often rely on publicly funded dental programs, which offer basic dental care to low-income families with children under 18. For example, Healthy Smiles Ontario offers free of charge dental care to children and adolescents 17 and under, if their household meets income eligibility requirements [Healthy Smiles Ontario, 2016]. The discontinuation of government oral health programs, for example in Ontario at age 18, may impact low-income adolescents and young adults, worsening health disparities among socioeconomic groups. Comprehensive oral healthcare strategies are essential to meet the evolving needs of individuals transitioning from adolescence to adulthood, especially for those from low-income backgrounds. Income and insurance influence the frequency of dentist visits, resulting in more visits from affluent [Schroth et al., 2015b] and privately insured groups [Quiñonez, 2014]. Canadians in lower-income households were less prone to seek dental care from a professional, regardless of whether or not they had dental insurance [Statistics



Canada, 2019] (Figure. 2.4). However, the introducing of the Canadian Dental Care Plan (CDCP) 2023 is expected to change the current situation. This new initiative will cover certain dental care expenses and improve access to dental care for uninsured Canadians with lower household incomes.



**Figure 2.4: Percentage of Canadians reporting a dental visit in the past year, by household income quintile and dental insurance coverage, population aged 12 and older in 2018. Source: [Statistics Canada, 2019]. Reproduced with permission from Statistics Canada.**

Data analysis from the Canadian Health Measures Survey 2007-2009 reveals that Canadians with lower or middle economic status lack preventive treatments and face delayed treatment interventions, leading to increased instances of decayed and missing teeth, periodontal disease, and oral pain [Ravaghi et al., 2013; Quiñonez, 2014]. This can be attributed to the increase in temporary and part-time employment, which reduces access to employment-based dental insurance, particularly for the lower- and middle-income groups of the Canadian population [Statistics Canada, 2019].

Addressing disparities requires policy changes, community initiatives, partnerships, and preventive measures. The recent establishment by the federal government of the “Canadian Dental Care Plan” for low-income Canadians is a step in the right direction. Additionally, comprehensive data is needed to fully understand oral health disparities among adolescents and young adults from low-income households. Such data can guide the development of targeted interventions and policies to effectively address these discrepancies.

### 2.3.3 Immigration and Marginalization

The government of Canada defines marginalized groups as underserved populations facing discrimination and integration difficulties, leading to high poverty rates. These groups often encounter barriers like limited access to oral healthcare, lower socioeconomic status, and discrimination, all contributing to worse oral health outcomes [de Arruda Mauricio and da Silveira Moreira, 2020]. These barriers may also result in negative perceptions of oral health and healthcare utilization, discouraging individuals from marginalized communities from seeking dental care, which in turn can exacerbate disparities in oral health outcomes [Bassim et al., 2020].

New immigrants often struggle to access oral healthcare due to its exclusion from universal coverage. The lack of adequate dental insurance coverage, coupled with limited financial resources, has proven to be a significant impediment to the acquisition of dental care for all Canadians [Bhatti et al., 2007; Azrak et al., 2017; Okechukwu et al., 2021]. However, these barriers are particularly significant for immigrant Canadians, resulting in less favorable utilization patterns for oral healthcare compared to the broader population.

A study by Mehra et al. (2019), examined the relationship between various socio-demographic factors and insufficient dental care utilization among immigrants in Ontario. The findings showed that around 33% of immigrants had not visited a dentist within the last year, when compared to 28% of “Ontarians” [Zangiabadi et al., 2017]. More than one quarter of immigrants sought dental services exclusively for emergencies, compared to 19% of “Ontarians” [Zangiabadi et al., 2017; Mehra et al., 2019]. According to the study findings, immigrants who are 18 years or older exhibit a higher likelihood of poor dental care utilization. The research further indicates that this trend is especially prevalent among individuals aged 18 to 34 years.

For a long time, Indigenous peoples in Canada have been facing significant inequalities in access to healthcare services, and this problem is particularly prominent in oral health [Hussain, 2022]. To tackle these existing disparities and challenges in oral healthcare faced by Indigenous communities, various initiatives and strategies have been put in place. One such initiative is the First Nations oral health strategy called "Teeth for Life". Nevertheless,

existing efforts have not been sufficient to fully close the oral health gap between Indigenous peoples and their non-Indigenous counterparts [Hussain, 2022]. In a comprehensive scoping review focusing on the provision and utilization of oral health services among Canada's Indigenous peoples, several key variables were examined. These encompassed affordability, accessibility, accommodation, acceptability of oral health services, individual characteristics, and the role of the public/government, all of which were found to influence oral health utilization among Indigenous peoples [Hussain, 2022].

Early childhood caries (ECC) refers to the presence of one or more decayed, missing, or filled teeth in a child under the age of 6-years. ECC affects a disproportionately high number of First Nations, Inuit, and Metis children, with almost 25% of them suffering from this severe condition. This alarming prevalence of ECC is a clear indication of social injustice and systemic inequities that are prevalent in our society. These children are more likely to face socio-economic challenges, which further exacerbate their dental health issues [Kyoon-Achan et al., 2021]. Therefore, ECC may have implications for oral health as Indigenous children transition into adolescence and young adulthood. Addressing oral health inequalities among marginalized groups involves not only improving access to oral healthcare services but also addressing the underlying factors that shape perceptions and behaviors related to oral health.

## 2.4 Theories Explaining Oral Health Inequalities

This section reviews the theoretical perspectives on how social economic status and biological factors may contribute to differential oral health outcomes in adolescents and young adults. Numerous theories and models have been proposed to gain a better understanding of the underlying causes of oral diseases, the pathways involved, and origins of disparities. In 1948, the WHO introduced a definition of health as "a complete state of physical, mental, and social well-being and not merely the absence of disease or infirmity" [World Health Organization, 2003]. Various health models have been proposed, including biomedical, behavioral, fundamental causes, and biopsychosocial models. Among these, the biomedical and biopsychosocial models of health are the most prominent and are described below.

### 2.4.1 Biomedical Model

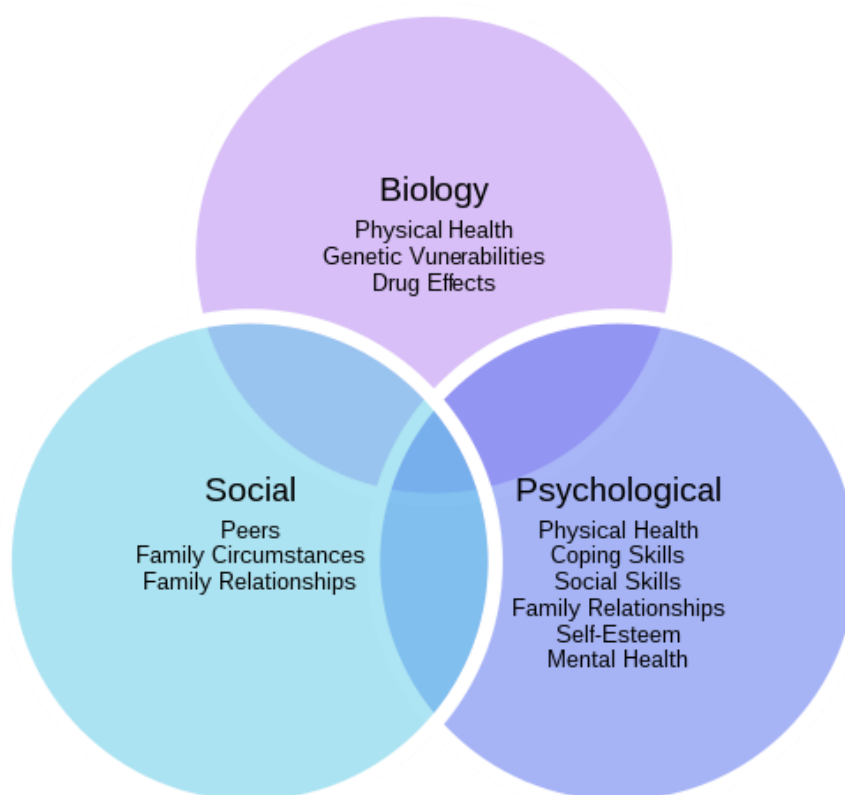
The biomedical model of health, which is also commonly referred to as the disease model, was first introduced in the 17th century. This occurred during a time when the significant scientific discovery of blood circulation was made by William Harvey [Hewa and Hetherington, 1995]. The biomedical model focuses primarily on the biological aspects of diseases and their treatment by medical interventions. While successful in managing acute conditions and guiding public health interventions, the biomedical model often overlooked social, psychological, and environmental factors, treating symptoms rather than underlying causes. In 1977, Engel put forth a noteworthy argument stating that the biomedical model failed to consider the psychological and social aspects of disease. This model solely relied on identifying the biological sources of illness, disregarding the various social determinants of health, which may also play roles in the development and progression of diseases [Engel, 1980].

In the context of oral health, the biomedical model treats the mouth as an isolated anatomical element separate from the whole body and the individual's mental state. Oral health prevention often targets high-risk behaviors without addressing the factors underlying these behaviors, such as stress and socioeconomic status [Watt, 2007]. Due to its restricted focus, the biomedical model fails to define health within a broader social context [Lu et al., 2015]. A more comprehensive model was needed to consider the patient's social context and the healthcare system to understand diseases better and to provide effective preventive strategies and treatments.

### 2.4.2 The Biopsychological Model

The biopsychosocial model of health complements the biomedical model by adding psychological and environmental influences (Figure. 2.5). In the paradigm of organismic hierarchy, the human being is regarded as the highest level of the hierarchy. However, when it comes to social hierarchy, humans are considered to be the lowest member [Engel, 1980]. The biomedical model focuses solely on the patient and their physical symptoms. On the other hand, the biopsychosocial model takes a more comprehensive approach and considers individual and environmental factors that can impact a person's health. In oral

health, this model shifts from the narrow approach of the biomedical model to consider broader determinants of health [Sabbah et al., 2007]. For instance, while the biomedical model attributes dental caries to the cariogenic bacterium *Streptococcus mutans*, it does not explain why some individuals do not develop caries despite having these bacteria present in the oral cavity. The biopsychosocial model views dental caries as a process influenced by biological (genetics, bacterial presence), psychological (stress), and social factors (socioeconomic status, diet, access to dental care). It connects oral health to an individual's physical and mental well-being, linking oral conditions to diseases beyond dental issues. This approach shifts from a disease-centered view to a focus on an individual's overall health and quality of life. Next, we explore the interconnections among socioeconomic, behavioral, psychological, and biological factors in health and disease.



**Figure 2.5: The biopsychosocial model of health which considers biological, psychological, and social factors. Reproduced with acknowledgment of Wikipedia and under Wikipedia Creative Commons Attribution-ShareAlike 4.0 International License.**

### 2.4.2.1 Socioeconomic Status

The biopsychosocial model emphasizes the role of various social determinants of health, including socioeconomic status, cultural background, family support, healthcare access, and social relationships. These determinants are critical in shaping health disparities and overall health outcomes. Socioeconomic status directly affects access to essential resources and opportunities, especially for those with lower socioeconomic status who may struggle with inadequate access to healthcare, poor nutrition, and unsafe living conditions. Education levels also impact health literacy and decision-making, further influencing health outcomes [Singh et al., 2019].

In the context of oral health, socioeconomic disparities consistently manifest across different strata of society, illustrating classic social gradients in health. Oral diseases serve as early indicators of overall population health and are sensitive clinical markers of social disadvantage [Bhandari et al., 2015]. Extensive literature has documented these social disparities, demonstrating that the evidence for social gradients remains consistent across various indicators, such as educational attainment, income, occupation, social class, and area-level socioeconomic status [Costa et al., 2012; Matsuyama et al., 2017; Baniyadi et al., 2021]. A systematic review conducted in 2015 explored the associations between socioeconomic status and dental caries experience, revealing that the association between low educational backgrounds and dental caries experience was significantly increased in countries with high Human Development Indexes, even after adjusting for confounding variables. Untreated carious lesions were associated significantly with low socioeconomic status [Schwendicke et al., 2015].

A subsequent study by Costa and colleagues updated these findings, establishing a connection between serious dental caries and lower socioeconomic status among adults residing in rich developing countries [Costa et al., 2018]. Research by Klinge and Norlund highlighted the correlation between disadvantaged socioeconomic circumstances and suboptimal periodontal health [Klinge and Norlund, 2005]. Additionally, a stable relationship has emerged between poor socioeconomic status and oral cancer, particularly in lower- and middle-income countries [Conway et al., 2008].

Although many studies have explored socioeconomic inequalities in dental caries within specific age groups, few have examined these disparities over a lifetime. For example, research from New Zealand found that the socioeconomic status of childhood was associated inversely with untreated dental caries [Poulton et al., 2002]. A Brazilian birth cohort study revealed that experiencing at least one stage of poverty from birth to age 15 has a harmful effect on dental caries experience, oral health-related behaviors, and dental service utilization by age 15 [Peres et al., 2007]. This study also identified that childhood poverty was linked to dental issues at age 24 [Peres et al., 2011].

Social factors in Engel's biopsychosocial model underscore the influence of socioeconomic status in shaping oral health disparities across the lifespan. The evidence highlights the importance of addressing social determinants, particularly socioeconomic factors, to reduce oral health inequalities and promote better overall health.

#### 2.4.2.2 Behavioral and Psychological Factors

Cultural, behavioral, and psychological pathways contribute to differences in health-related behaviors among socioeconomic groups. Extensive research consistently demonstrates that individuals with lower SES engage in health-risk behaviors more than those with higher SES, leading to increased illness rates. Factors like smoking, alcohol consumption, poor oral hygiene, and excessive sugar and carbohydrate intake contribute to oral diseases and inequalities [Levin et al., 2015]. Changes in behavior during the transition from childhood to adulthood can have longstanding effects on oral health. For instance, sugary snacks and soft drinks increase the risk of developing caries, while tobacco use is linked to an increased chance of periodontal diseases [Nociti et al., 2015; Thang Le et al., 2021].

However, it is crucial to consider that behavioral factors are not solely determined by personal choices; they are profoundly influenced by the social context. This perspective aligns with the psychosocial explanation, emphasizing the crucial role of social networks in providing opportunities for social support that contribute to improved health outcomes [Sanders et al., 2006].

The psychosocial theory further argues that health disparities result from variations in psychological distress experienced by different socioeconomic groups. Factors such as financial hardship, limited social support, and adverse life events may directly impact oral health through neurobiological pathways [Sisson, 2007; Sabbah et al., 2009a]. Indirectly, such distress could lead to poor oral hygiene and irregular dental care [Berkman et al., 2014]. Individuals living in adverse social conditions are often vulnerable to high levels of psychological and physiological stress, resulting in poor oral health outcomes. Stressful life experiences can arise from factors such as low income, inadequate accommodation, insufficient food, poor and unstable working conditions, and various forms of discrimination based on identity, race, gender, or disability [Sabbah et al., 2009b; Singh et al., 2019]. Behavioral, psychological, and social factors all play a role in oral health disparities. Understanding these dynamics is crucial to address inequalities and promote better overall health.

#### 2.4.2.3 Biological Factors

The biopsychosocial model provides a comprehensive framework for understanding how adverse socioeconomic exposures and psychological pathways lead to the development of disease and illness. Even though the link between social status and oral health has been well-studied, the concept of biological factors in oral health inequality has received less attention [Solís, 2018; Tsakos et al., 2023].

Growing evidence supports that exposure to chronic and acute stressors over a lifetime can have detrimental health effects [Gomaa et al., 2016]. These stressors can lead to physiological dysregulation and contribute to disease development directly through biological pathways or indirectly by promoting health-damaging behaviors [McEwen and Stellar, 1993]. Chronic stress, often arising from adverse social and socioeconomic conditions, can lead to changes in behavior and biology, negatively affecting the immune system, hormone regulation, and overall health, and increasing disease sensitivity [Blane et al., 2013; Gomaa et al., 2020]. Several studies have highlighted the relationship between stress and various health conditions, such as cardiovascular, metabolic, immune, and neuroendocrine disorders. For example, research indicates that social determinants of health are linked to cardiovascular diseases through mechanisms like the overproduction



of stress hormones, inflammation, and disruptions in immune cell function [Powell-Wiley et al., 2022]. Additionally, adverse childhood experiences can lead to long-lasting epigenetic changes, increasing the risk of conditions like diabetes, heart disease, and lung disease [Mehta et al., 2013].

Regarding oral health disparities, some studies have shown an association between stress and oral health, although limited attention has been given to the biological correlates of stress. Periodontal diseases depend on the efficiency of the innate immune response to bacterial pathogens, while dental caries is influenced by host factors, including immune components and other biological factors [Ji et al., 2007; Gomaa et al., 2016]. A study by Boyce et al. found that socioeconomic position, HPA axis dysregulation, and cariogenic bacteria were distinct and independent factors linked to dental caries in children [Boyce et al., 2010].

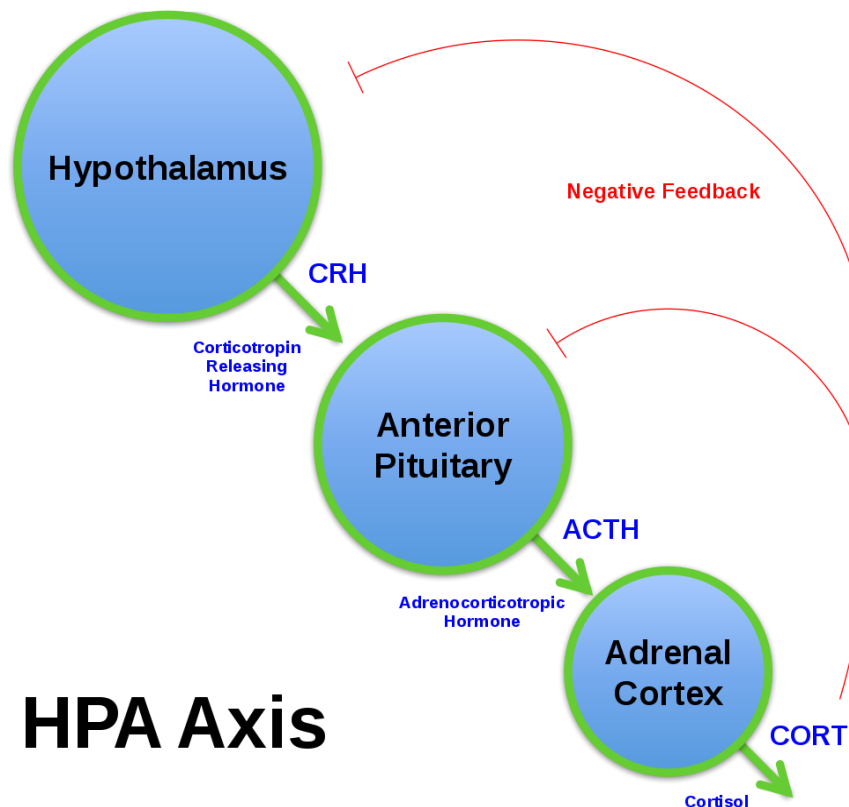
A cross-sectional study of 200 students (15 to 19 years old) examined the relationship between stress and self-perceived oral health status. The study found that a significant portion of participants experienced stress, showed signs of bruxism, had dental decay, and reported gingival problems [Arman et al., 2016].

Nelson et al. demonstrated how parental intrinsic and extrinsic psychosocial factors associated with oral behaviors and dental caries in children and adolescents [Nelson et al., 2012]. Additional studies revealed associations between adolescents' psychosocial factors – like oral health beliefs and self-esteem, as well as parental psychosocial factors – with oral health-related behaviors and gingival disease [Sfreddo et al., 2019]. Lastly, a study of Japanese medical students indicated a substantial effect of chronic educational stressors on mental health and suggested an association between expression of glucocorticoid receptor  $\beta$  and the response to psychological stress [Kurokawa et al., 2011].

In summary, this body of evidence underscores how stress can affect oral health through biological mechanisms, including immune and inflammatory responses. Measuring biological markers of stress could be useful in preventing and addressing oral diseases at both societal and individual levels.

## 2.5 The Hypothalamic-Pituitary-Adrenal (HPA) Axis and Stress Responses

The hypothalamic-pituitary-adrenal axis is a complex network includes the hypothalamus, pituitary gland, and adrenal glands. The HPA axis plays a crucial role in regulating the body's physiological response to stress by coordinating with the sympathetic nervous system [Charmandari et al., 2005]. When the hypothalamus detects a stressor, it secretes corticotrophin-releasing hormone (CRH). CRH then signals the pituitary gland to release adrenocorticotrophic hormone (ACTH), which in turn stimulates the adrenal glands to produce cortisol, a primary glucocorticoid in humans [Raul et al., 2004] (Figure. 2.6).



**Figure 2.6: HPA-axis regulation.** Schematic representation of HPA-axis activation depicting the cascade of events leading to the release of glucocorticoids (cortisol) and the connections through which negative feedback regulates the HPA-axis. Reproduced with acknowledgment of Wikipedia and under Wikipedia Creative Commons Attribution-ShareAlike 4.0 International License.

The concept of 'homeostasis,' introduced by Walter Cannon in the 20th century, describes the maintenance of physiological equilibrium essential for an organism's survival [Cannon and Pettit, 1926]. Threats to this balance can activate the 'fight or flight' response. Hans Selye coined the term 'stress' to “the nonspecific response of the body to any demand” [Selye, 1975]. The field of stress research is divided into biological, psychological, and sociological traditions. Stress is challenging to define due to its varied usage across different fields. A cumulative approach is most suitable for comprehending the entire stress process.

Recent evidence has strengthened the link between stress and disease. Several factors such as anxiety, social loneliness, over-demanding life events, and lack of control can accumulate in a person's life, increasing the probability of various health issues. Childhood stress has been associated with increased susceptibility to psychological and physical health problems in adolescence and adulthood, involving lung disease, heart disease, diabetes, cancer, depression, and premature mortality [Anda et al., 2009; Rich-Edwards et al., 2010].

Chronic stress weakens the immune system, making the body more susceptible to oral infections [Gomaa et al., 2016]. Stress can also lead to unhealthy behaviors that increase the risk of dental diseases and contribute to bruxism, causing tooth damage, sensitivity, and jaw pain. It can also decrease saliva production, causing dry mouth and raising the probability of dental caries and periodontal disease [Bosch et al., 1996; Tikhonova et al., 2018]. Most research emphasizes the significant role of early-life adversity in shaping long-term health outcomes. The pathways connecting environmental stressors to health disparities are complex, including adverse health behaviors and physiological dysregulation. This complexity highlights the significance of implementing proactive procedures to aid children who are at risk and enhance the effectiveness of treatment methodologies. Studying the impact of early stress could provide approaches to enhance the health of children and future adults. In response to psychological or physical stress, the autonomic nervous system (ANS) and HPA axis prepare the body to cope with environmental demands. Responses that are excessively active, are insufficient, fail to

habituate, or recover inefficiently are considered maladaptive reactions that may contribute to the development and progression of disease [McEwen and Stellar, 1993].

## 2.5.1 Regulation of the HPA Axis

Regulating the HPA axis is important in daily life as cortisol plays a vital role in the control of various physiological processes such as fat and glucose metabolism, blood pressure, immune responses, and inflammatory reactions. The ability of the HPA axis to adapt to environmental factors is crucial for maintaining proper bodily and brain function. In the following sections, we will explore the three main regulatory mechanisms.

### 2.5.1.1 Chronobiological Pattern

Cortisol levels in healthy individuals follow ultradian, circadian, and seasonal rhythms. Pulsing secretion of corticotropin-releasing hormone from hypothalamus considered as a ultradian rhythm occurs every 1-2 hours. This pulsing secretion is hypothesized to prevent the HPA axis from downregulating, which maintains its ability to respond to stress [Laudat et al., 1988]. These pulses vary among individuals and can change due to physiological events, such as puberty, lactation, and aging. The timing of glucocorticoid release also follows a circadian pattern, with a gradual increase in levels during the early morning hours. The peak of this release typically occurs around 30 to 40 minutes after awakening following which levels gradually decline throughout the rest of the day, until they reach their lowest levels around midnight. In normal subjects, salivary cortisol levels range from 10.2–27.3 nmol/L at 8:00 AM, falling to 2.2–4.1 nmol/L at 8:00 PM [Laudat et al., 1988]. Cortisol seasonal rhythms refer to variations in cortisol levels that occur over the course of a year, typically in response to changes in environmental factors such as daylight exposure and temperature. Certain research studies have reported that cortisol levels could be higher during the summer/ or winter. However, some studies failed to provide evidence of any seasonal effect on cortisol levels [Persson et al., 2008].

### 2.5.1.2 Negative Feedback

In addition to stimulatory regulation, the HPA axis is subject to inhibitory control mechanisms, which involve negative feedback. Negative feedback is activated in response

to circulating cortisol levels, effectively suppressing the production of CRH and ACTH, consequently reducing the production and secretion of cortisol (Figure. 2.6). The HPA axis exhibits three distinct types of negative feedback mechanisms. The first mechanism, known as rapid negative feedback, occurs within seconds to minutes of changes in glucocorticoid concentration in the bloodstream. It is often observed during stressful events or steroid administration, leading to immediate inhibition of ACTH production. The second mechanism, delayed or intermediate feedback, takes approximately 30 minutes to hours to influence pituitary and hypothalamic cell responses. The reduction in CRH and ACTH secretion is proportional to the prior concentration of glucocorticoids achieved in the plasma. The third mechanism, known as slow feedback, occurs over days to weeks of continuous glucocorticoid exposure and affects basal and stimulated pituitary and hypothalamic function [Russell et al., 2012].

### 2.5.1.3 Stress

Stress is another mechanism which regulates the HPA axis. When an individual is exposed to physical or psychosocial stressors, such as infection, pain, or fear, the HPA axis is activated to retain the necessary adaptive ability to manage the stressor. The stress response is comprised of three phases: stress reaction, recovery, and adaptation [Oitzl et al., 2010]. Two classes of hormones are released in response to various stressors: catecholamines and glucocorticoids. The rapidity and intensity of both reactions depend on the stressor's nature. Catecholamines, including noradrenaline and adrenaline, act via the nervous system, triggering immediate physical reactions associated with the fight-or-flight response. In contrast, glucocorticoids, such as cortisol, act via an endocrine pathway and support the effects of catecholamines over minutes or hours [McEwen, 2003]. The release of cortisol is terminated via the negative feedback loop described above.

During the recovery, the stress reaction is contained, and the information is encoded. In the adaptive phase, the experience is consolidated in the memory to promote adaptive behavior in the future. However, if the stress response is chronically stimulated, adaptation can become maladaptive and potentially more harmful than the initial stressor, mainly if it is primarily psychological [Walker et al., 1997].

### 2.5.2 Dysregulation of the HPA Axis

Dysregulation of HPA axis can vary in its severity, ranging from mild to severe dysregulation, and caused by endogenous and exogenous factors. Endogenous dysregulation can be either hypocortisolism (Addison's Disease) or hypercortisolism (Cushing's Disease). The researchers have identified significant features associated with this condition in stress-related disorders. These fundamental characteristics encompass diminished cortisol levels under baseline conditions, attenuated adrenocortical responsiveness when exposed to stressors, and heightened negative feedback sensitivity within the HPA axis [Heim et al., 2000].

HPA-axis hyperactivity or hypercortisolism is characterized by increased secretion of adrenocorticotrophic hormone along with elevated levels of cortisol, and reduced responsiveness to negative feedback [Nemeroff, 1996; Heim et al., 2000]. This condition can have a significant impact on an individual's mental and physical health, and is often associated with symptoms such as anxiety, depression, and sleep disturbances. Understanding the underlying mechanisms of HPA-axis hyperactivity and hypercortisolism can help in the development of effective interventions for individuals struggling with stress-related disorders.

HPA axis dysregulation can also be caused by exogenous factors, such as the glucocorticoids administered for their immunosuppressive and anti-inflammatory effects, and for adrenal insufficiency.

In conclusion, understanding the HPA axis and its potential dysregulation is critical for identifying and treating various medical and psychiatric conditions.

### 2.5.3 Measurement of Cortisol

Cortisol exists in three forms in the blood: free cortisol, protein-bound cortisol, and cortisol metabolites [Mendel, 1989]. Most of the cortisol is bound to plasma protein, with more than 80% bound to cortisol-binding globulin and 10-15% to albumin. Only the small portion of cortisol, around 5%, remaining free is considered biologically active [Lewis et al., 2005]. Total cortisol levels increase during stress, and changes in corticosteroid-binding

capacity and affinity can alter the proportion of free cortisol, impacting its physiological effects [Cameron et al., 2010].

Steroid hormones such as cortisol can cross cell membranes because of their fat-soluble properties. Traditionally, physiological stress levels have been assessed by measuring cortisol levels in blood, saliva, or urine. Such measurements are effective for evaluating short-term stress responses. In the absence of stress, cortisol levels peak within the first 15 to 30 min after waking and then gradually decline over the subsequent 24 h, only to rise again upon the next waking. During a state of stress, the HPA axis releases additional cortisol to cope with the stressor, resulting in concentrations higher than baseline levels, with a peak occurring approximately 15 to 30 min after encountering the stressor. However, when it comes to assessing prolonged or chronic stress, these conventional measurements of cortisol in blood, saliva, and urine may not provide accurate results due to significant variability arising from individual characteristics and environmental factors. This is where studies on hair cortisol come into play, as they offer insight into a different aspect of the HPA axis. Hair cortisol measurements reflect long-term total cortisol exposure, spanning months to years, providing a measure of the cumulative impact of stress over extended periods.

### 2.5.3.1 Measurement of Short-term Changes in Cortisol

Short-term changes in cortisol can be measured in blood, saliva, and urine. The measuring of salivary cortisol has become a widely used tool in stress studies offering advantages over blood or urine measurements [Kirschbaum and Hellhammer, 1994]. The noninvasive nature of saliva collection and the capability of obtaining samples at different points of time for different periods of time is useful for stress researchers [Kambalimath et al., 2010; Pani et al., 2013; Shafi et al., 2021].

Cortisol levels in saliva are closely correlated with the free cortisol levels in the blood. The correlation between salivary and unbound blood cortisol levels is usually so strong that it any fluctuations in salivary cortisol levels can be attributed to corresponding changes in unbound blood cortisol levels [Kirschbaum and Hellhammer, 1994]. This correlation is

primarily because cortisol enters the salivary gland cell and the oral cavity mainly by passive diffusion.

However, it is important to note that salivary cortisol levels exhibit substantial variations both within and between individuals. These variations in levels can be attributed to several factors, including circadian rhythm, pulsatile secretion, and responses to acute transient stressors like nervousness [Kidambi et al., 2007]. Inherent differences among individuals and fluctuations within individuals over time can be considered confounding variables that complicate the comparison of findings across different studies. In addition, these measurements poorly reflect long-term exposure to stress.

### 2.5.3.2 Measurement of Long-term Changes in Cortisol

The constraints related to short-term cortisol measurements have prompted researchers to investigate the potential of measurement of long-term changes. As a result, cortisol levels in human hair have been identified as a viable option, with initial reports appearing in 2000 and 2004 [Cirimele et al., 2000; Raul et al., 2004]. It has been established that cortisol is absorbed into hair via diffusion from the capillary circulation of blood that supplies the hair roots. It is worth noting that hair typically grows at a rate of approximately 1 cm per month. This growth rate allows for the accumulation of cortisol within the hair as it elongates, reflecting cortisol levels during preceding months [Greff et al., 2019].

The process of extracting cortisol in hair involves the use of methanol and subsequent analysis by means of immunoassays [Luz et al., 2003] or liquid chromatography tandem-mass spectrometry (LC-MS/MS) [Cirimele et al., 2000; Zhang et al., 2018]. While immunoassays, such as radioimmunoassay (RIA) or (ELISA), are restricted to assessing only one steroid [Russell et al., 2012], LC-MS/MS is capable of measuring numerous steroids in a single sample, thus facilitating the development of steroid profiles.

Studies examining the correlation between hair cortisol levels and saliva cortisol concentrations have likewise generated mixed results. While some studies have found consistency between hair and salivary cortisol levels [Zhang et al., 2018; Sääksjärvi et al., 2021], others have not observed a strong correlation [Cruickshank et al., 2021]. These



inconsistencies highlight the intricate nature of the stress response and the need for further research into factors influencing the relationship between hair cortisol and salivary cortisol.

Studies have shown association between hair cortisol and self-reported stress-related measures [Lynch et al., 2022]. One such measure is the Perceived Stress Scale (PSS), a self-reported questionnaire that assesses the extent to which individuals perceive their lives to be unpredictable, uncontrollable, and overloaded with stress [Cohen et al., 1983].

## 2.6 Glucocorticoid Receptors and Signaling

Glucocorticoids modulate many metabolic, cardiovascular, immune, and behavioral functions. Glucocorticoid activity is mediated by two nuclear receptors [Jurueña et al., 2004; Smith and Vale, 2006]. The high-affinity mineralocorticoid receptor (MR) responds primarily to normal levels of glucocorticoids and helps regulate the HPA axis. Conversely, the low-affinity glucocorticoid receptor (GR) is activated by elevated levels of glucocorticoids and plays a role in reducing these levels to restore balance to the HPA axis [Kadmiel and Cidlowski, 2013; Panagiotou et al., 2021]. Therefore, when glucocorticoids are high, the GR may be more important in managing stress responses effectively. Prior to activation, the MR and GR are situated within the cytoplasm. Upon activation, these receptors translocate to the nucleus. This process leads to their binding to specific DNA sequences, which are referred to as the MR and GR response elements. Alternatively, these receptors may engage with other DNA-bound transcription factors, facilitating either the transactivation or transrepression of target genes [Gjerstad et al., 2018].

## 2.7 Expression of Glucocorticoid Receptor Genes

The human glucocorticoid receptors (GRs) are encoded by the nuclear receptor subfamily 3 group C member 1 (NR3C1) gene situated on chromosome 5 (5q31.3) [Turner and Muller, 2005]. This gene is comprised of 9 exons. Exon 1 is an untranslated region (UTR), exon 2 encodes for N-terminal domain (NTD), exon 3 and 4 for DNA- (DBD), and exons 5-9 for the hinge region and ligand binding domain (LBD). Additionally, the GR gene encompasses two terminal exons, 9 $\alpha$  and 9 $\beta$ , which undergo alternative splicing to produce GR $\alpha$  and GR $\beta$  [Smith and Vale, 2006; Panagiotou et al., 2021].

GR $\alpha$ , which is composed of 777 amino acids, is the most prevalent form of GR and functions as a conventional ligand-dependent transcription factor. In contrast, GR $\beta$ , which comprises 742 amino acids, lacks a significant portion of the ligand-binding domain and is thought to negatively regulate GR $\alpha$  by acting as a dominant negative inhibitor [Panagiotou et al., 2021]. Since these isoforms of GR have very different functional properties, examining the expression of GR isoforms is vital.

GR $\alpha$  expression has been observed in various cell and tissue types. When the ligand is absent, GR $\alpha$  predominantly resides in the cytoplasm, where it binds to heat-shock proteins, rendering it inactive. To become biologically active, cortisol must pass through the cell membrane and bind to GR $\alpha$  [Saeedfar et al., 2018]. Upon binding, GR $\alpha$  undergoes phosphorylation, dissociates from heat-shock proteins, and translocates to the cell nucleus. Within the nucleus, ligand-activated GR $\alpha$ , functioning as a dimer, interacts with DNA at glucocorticoid response elements (GREs) [DeRijk et al., 2002; Bray and Cotton, 2003]. Furthermore, GR $\alpha$  can interact with other transcription factors, acting as either a monomer or dimer, to exert inhibitory effects on the transcription of inflammatory genes. This inhibition leads to the suppression of the production of pro-inflammatory cytokines and adhesion molecules. In individuals with bipolar disorder or major depression, there was a notable decrease in GR $\alpha$  mRNA levels, while GR $\beta$  expression remained unchanged compared with normal control subjects [Kurokawa et al., 2011]. Furthermore, a clear link was established between life stress and reduced GR $\alpha$  expression in leukocytes among children with asthma, and with metabolic and inflammatory diseases [Chen, 2006; Panagiotou et al., 2021] .

The expression of GR $\beta$  is significantly lower in comparison to GR $\alpha$  and its inability to bind to glucocorticoids renders it transcriptionally inactive [Panagiotou et al., 2021]. Immunohistochemical studies have detected GR $\beta$  expression primarily in specific cell types, particularly inflammatory cells. While the exact physiological role of GR $\beta$  remains unclear, its overexpression has been observed in conditions associated with glucocorticoid resistance, including asthma, ulcerative colitis, chronic lymphocytic leukemia, and nasal polyposis [Saeedfar et al., 2018].

In the context of analysis of GR expression, several studies have focused on blood-derived RNA sources. Nonetheless, drawing blood necessitates trained professionals, specialized equipment for collection and storage, and can cause discomfort for the individual. Therefore, finding different RNA sources for transcriptomic analysis would be helpful. This raises the question of whether saliva might present a more acceptable alternative than blood.

## 2.8 RNA Extraction Method from Whole Human Saliva

Human saliva is a multifaceted fluid derived from major and minor salivary glands, constituting approximately 99% water. Its composition includes a variety of electrolytes, mucus, white blood cells, and epithelial cells, making it a source for DNA and RNA extraction.

Recognized as a promising reservoir of biomarkers for diagnostics, saliva exhibits distinct changes in its biomolecular composition associated with various diseases [Pandit et al., 2013; Gandhi et al., 2020]. Often referred to as the "mirror of the body," saliva closely mirrors blood composition, encompassing hormones, enzymes, antibodies, and genetic materials transferred from the bloodstream through mechanisms like ultrafiltration, diffusion, paracellular routes, and active transport [Park et al., 2006; Palanisamy and Wong, 2010].

RNA found in the oral cavity originates from multiple sources, including saliva secretion from major salivary glands, gingival crevicular fluid, and oral epithelial cells [Park et al., 2006]. Salivary gland secretions may carry RNA from acinar cells or circulating sources. Gingival crevicular fluid is another RNA source, as it releases numerous blood cells into the oral cavity. Mucosal epithelial cells within the oral cavity and micro-wounds can also contribute to the presence of RNA [Delima and Van Dyke, 2003].

This diverse range of sources highlights saliva's potential as a non-invasive and accessible medium for extraction and analysis of RNA to monitor health and diagnose various conditions.

Preservation of the integrity of salivary RNA is a critical requirement in downstream transcriptomic analysis due to its vulnerability to degradation by RNases and other nucleases present in saliva. To overcome this challenge, significant progress has been made in developing proper collection and preservation techniques. The use of stabilizing reagents or snap-freezing is essential for preserving RNA integrity during sample processing [Gandhi et al., 2020].

Various methods exist for isolating RNA from saliva, including the use of phenol and guanidinium isothiocyanate, commercially available silica membrane spin columns, or magnetic bead-based RNA isolation kits [Ostheim et al., 2020]. However, many of these protocols are relatively expensive and may yield limited amounts of RNA.

The existing literature of salivary RNA extraction mainly focuses on utilizing the cell-free salivary supernatant as the RNA source, as opposed to the salivary cellular pellet, which often requires larger volumes of saliva [Dietz et al., 2011]. This underscores the need for a cost-effective, high-throughput RNA isolation method that can produce sufficient RNA from both the cell-free salivary supernatant and the cellular pellet, even when working with small saliva volumes [Gandhi et al., 2020; Ostheim et al., 2020]. Addressing this need would contribute to improving the efficiency and accessibility of salivary RNA analysis.

## 2.9 Summary

The relationship between stress and caries development is complex and requires a comprehensive understanding of various bodily systems and the social and psychosocial environment. Stress is known to affect the immune system, which in turn can impact oral health outcomes. Additionally, stress can lead to changes in eating habits, which may increase caries risk. The pathways through which stress affects oral health outcomes have yet to be fully understood, and further research is needed to establish a conclusive connection. Thus, understanding the interplay between stress and oral health outcomes is essential in identifying contributing factors and potential pathways involved in the development of caries.

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## Chapter 3

### 3 Rationale, Objectives and Hypotheses

Although there have been plenty of advancements in oral health, disparities in oral diseases and their treatment persist and are a significant public health issue [Pitts et al., 2011; Peres et al., 2019]. These inequalities are often associated with social stratification, linked to factors such as income, insurance, education, area of residence, age, and gender [Peres et al., 2005]. However, there is a gap in studies investigating the biological factors that may be associated with these inequalities and differential oral health outcomes [Watt, 2005; Gomaa, 2022]. This gap may be attributed to various reasons, including multidisciplinary challenges and the multifactorial causes of oral diseases [Sheiham and Watt, 2000; Pitts et al., 2017]. A multidisciplinary approach is essential in studying oral health outcomes due to the involvement of various biological, behavioral, psychological, social, and environmental factors [Finlayson et al., 2010]. Identifying pathways associated with inequalities may enhance understanding and facilitate the planning of targeted interventions to reduce oral health inequalities.

Monitoring oral health is considered crucial across various age groups, yet research has often focused on young children [Brukienė and Aleksejūnienė, 2009]. Understanding the extent to which social diversity and psychological factors may influence the oral health of adolescents and young adults requires further exploration [Baker et al., 2010]. In Ontario, where universal healthcare plans have not previously covered dental care, low-income residents often lost enrollment in the government assistance program, Healthy Smiles Ontario, upon reaching 18 years of age [Healthy Smiles Ontario, 2016]. However, it is anticipated that the situation will improve with the introduction of the Canadian Dental Care Plan (CDCP). This new initiative is expected to cover some dental care costs and improve dental care accessibility for low-income Canadians.

Recent evidence suggests that stress can negatively impact oral health in terms of dental caries and periodontal diseases, and might contribute to oral health inequalities [Boyce et al., 2010; Tikhonova et al., 2018; Gomaa et al., 2020]. This perspective arises from the knowledge that exposure to stressors has potential health-damaging effects. The influence

of stressors on oral health is intricate, as they can either act directly by promoting disease progression via biological pathways or act indirectly by inducing unhealthy behaviors [Tikhonova et al., 2018]. Chronic exposure to stress during adolescence can lead to alterations in the HPA axis, which is crucial in regulating stress responses [Van den Bos et al., 2014; Harris et al., 2017]. These changes can further impact emotional responses and cognitive functions, affecting overall and oral health outcomes [Lenz, 2001].

Patients with periodontitis are known to experience a significant increased levels of cortisol in their saliva and gingival crevicular fluid [Akcali et al., 2013]. This hormone is part of the body's natural response to inflammation and stress [Rosania et al., 2009], which may introduce a level of ambiguity when measuring cortisol levels associated with dental caries experience. Hence, in our studies, we excluded patients with periodontal disease from such measurements to ensure their accuracy and reliability.

Although researchers have used cortisol levels to assess activation of the HPA axis, limitations of this approach have been documented. Recently, it has been suggested that quantifying expression of the glucocorticoid receptor (GR) may overcome some of the limitations of measuring cortisol alone [Vassiliou et al., 2019]. The binding of cortisol to the GR influences gene expression and induces negative feedback that brings cortisol back to normal levels [Pariante and Miller, 2001; Stephens and Wand, 2012]. Variations in GR gene expression may be one factor causing dysregulation of the HPA axis dysregulation. These changes may impact the HPA response to stress by impairing negative feedback regulation of cortisol release [Pariante and Miller, 2001].

Building upon this foundation, the overall objective of my research was to study the associations among stress, socioeconomic status, demographic factors, and oral health outcomes in adolescents and young adults. These studies may expand our knowledge of the pathways underlying inequalities in oral health.

**Project 1: Neighborhood-Level Inequalities in Dental Care of Adolescents and Young Adults in Southwestern Ontario**

Objective: Examine whether the association of neighborhood-level socioeconomic status with the cost of dental care and dental care outcomes differs between adolescents (15-17 years) and young adults (18-24 years).

Hypothesis: There are socioeconomic differences in dental treatment utilization in adolescents and young adults.

**Project 2: Relationships among Cortisol, Perceived Stress, and Dental Caries Experience in Adolescents and Young Adults**

Objective: Assess the association among perceived stress, cortisol levels (in hair and saliva), and the overall caries experience of adolescents and young adults aged between 15 and 25 years.

Hypothesis: Individuals with good oral health outcomes will show lower cortisol levels (in hair and saliva) compared to individuals with poor oral health outcomes in a sample controlled for socioeconomic factors.

**Project 3: Profiling mRNA encoding glucocorticoid receptor  $\alpha$  in saliva: Relationship to hair cortisol levels in individuals aged 15-25 years**

Objectives:

- A. Compare GR $\alpha$  transcript levels between individuals with and without dental caries experience.
- B. Compare GR $\alpha$  transcript levels between individuals with and without elevated hair cortisol levels.
- C. Compare GR $\alpha$  transcript levels among the following groups of individuals: a) with dental caries experience and normal hair cortisol levels; b) without dental caries experience

and normal hair cortisol levels; c) with dental caries experience and elevated hair cortisol levels; and d) without dental caries experience and elevated hair cortisol levels.

Hypothesis: The expression of glucocorticoid receptors will be negatively associated with long-term stress and will be negatively associated with dental caries experience among adolescents and young adults.

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## Chapter 4

### 4 Neighborhood-Level Inequalities in Dental Care of Adolescents and Young Adults in Southwestern Ontario<sup>1</sup>

This chapter has been adapted from the paper titled “Neighborhood-Level Inequalities in Dental Care of Adolescents and Young Adults in Southwestern Ontario” with permission of the publisher (see Appendix 9).

#### 4.1 Chapter Summary

We examined whether the association of neighborhood-level socioeconomic status (SES) with the cost of dental care and dental care outcomes differs between adolescents and young adults. A total of 2915 patient records were split into two groups: adolescents (15 to 17 years of age) and young adults (18 to 24 years of age). Three dental care outcomes, routine oral evaluation (OEV-CH-A), utilization of preventive services (PRV-CH-A), and dental treatment services (TRT-CH-A) were determined according to the Dental Quality Alliance (DQA) criteria. Associations of neighborhood SES and other sociodemographic variables with dental care outcomes and the cost of dental care were assessed using binary logistic and univariate linear regression models, respectively. Young adults had significantly lower PRV-CH-A and higher TRT-CH-A scores when compared to adolescents. We observed a significant negative association between TRT-CH-A and median household income in both adolescents and young adults. Utilization of dental treatment services was positively associated with the cost of care in both age groups, whereas utilization of preventive services was inversely associated with the cost of care in young adults, but not in adolescents. Neighborhood-level income was inversely associated with increased TRT-CH-A in both young adults and adolescents. In summary, young adults showed significantly worse preventive and treatment outcomes when compared to

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<sup>1</sup> A version of this chapter has been published (Abouseita N, Gomaa N, Dixon SJ, Pani SC. Neighborhood-level inequalities in dental care of adolescents and young adults in Southwestern Ontario. *Children (Basel)*. 2022;9(2):183).



adolescents. Moreover, individuals from neighborhoods with a lower household income showed a significantly higher cost of dental care, yet worse treatment outcomes.

## 4.2 Introduction

The transition from adolescence to adulthood is marked by several changes that could impact the health and well-being of individuals [Wilens and Rosenbaum, 2013]. The oral health of adolescents and young adults has recently begun to receive attention in literature. Although dental caries is the most common health problem for adolescents [Percy, 2008; Holst and Barzel, 2012] research suggests that adolescents are also at a risk of other oral diseases, such as traumatic dental injuries and periodontal diseases [Percy, 2008]. Individuals who suffer from higher levels of dental caries in childhood may be also more prone to developing dental problems in adulthood, a fact that supports the need to study dental caries and dental care outcomes as children transition to adolescence and adulthood [Manton, 2018].

In addition to being influenced by genetics and health behaviors, several social and economic changes that occur as one transitions from adolescence to adulthood can also impact oral health [Baker et al., 2010; Park et al., 2022]. For example, in Ontario, children under the age of 18 years who are from low-income households are eligible for government-funded dental care through the Healthy Smiles Ontario (HSO) program [Healthy Smiles Ontario, 2016], which essentially means that adolescents (15 to 17 years of age) from low-income households can access dental care through this program, whereas young adults (18 to 24 years of age) cannot.

Worldwide, the cost of treatment of oral diseases, whether paid for by public or private insurance or by the patient, can be a barrier to accessing dental care for those with limited financial means [Farmer et al., 2016]. Canada's oral health care system is largely privatized; approximately 60% of expenditures are financed by private insurance, such as employer or individual plans, and 35% are paid directly by the patient. Theoretical models for caries risk prediction, based on social and demographic variables, show that these barriers are borne disproportionately by socially disadvantaged populations [Díaz Rosas et al., 2018; Obadan-Udoh et al., 2019]. Income-related inequalities persist even when

patients have dental insurance coverage and good oral hygiene practices [Duncan and Bonner, 2014]. Canadians living in households with lower incomes are less likely to visit a dentist, with the proportion reaching 49.6% amongst those with low income and without insurance [Statistics Canada, 2019]. The association between socioeconomic status (SES) and oral health has been widely studied, however there has been little research on how differences in SES influence oral health in specific age groups. Although there has been some research using advanced methods to explore oral health inequality in Canada, there is little information on the magnitude of inequalities in adolescents and young adults, or on the possible impact of the loss of government-funded programs at 18 years of age. The cost of dental care is often a reflection of the treatment sought, and billing data has been shown to be an indicator of both access to dental care and the quality of dental care outcomes [Levitin et al., 2019].

Although advances in technology have led to rapid progress in the field of data mining [Breault, 2017; Levitin et al., 2019], it has not been used widely to interrogate Canadian oral health billing databases. Protocols for collecting electronic dental billing data have been proposed by the Dental Quality Alliance (DQA) and have been used successfully to measure the quality of dental care received [Ojha and Aravamudhan, 2016; Breault, 2017]. Neighborhood-level sociodemographic data in Canada are available at the level of forward sortation area (FSA) postal codes [Statistics Canada, 2018], which have been used by both diabetes and cancer researchers as a measure of neighborhood-level socioeconomic status [Musa et al., 2013; Hipp and Chalise, 2015]. There is some data on the mapping of dental caries according to postal codes in children [Holmén et al., 2018], and the relationship between dental billing data and neighborhood-level sociodemographic variables among Canadian children has recently been explored [Podskalniy et al., 2020]. The present study examined whether the association of neighborhood-level socioeconomic status (SES) with the cost of dental care and dental care outcomes differs between adolescents and young adults.

## 4.3 Methodology

### 4.3.1 Ethics Approval

This research was approved by the Health Sciences Research Ethics Board (HSREB) at the University of Western Ontario (2020-115567-37532) and the use of secondary data was conducted within the principles and guidelines of the Canadian Tri-Council Policy Statement on Ethical Conduct for Research Involving Humans [McDonald, 2001].

### 4.3.2 Screening of Patient Records

The present study utilized secondary billing data from the records of patients visiting the dental clinics of the Schulich School of Medicine & Dentistry at the University of Western Ontario in London, Ontario. Electronic dental records of patients aged 15–24 years at the time of the last dental visit were screened. Patients who had at least one additional treatment code within a 180-day period of the first recorded visit for each year studied were included in the study according to the protocol set by the DQA to ensure that there is no skew or bias [Ojha and Aravamudhan, 2016]. Only FSA codes with at least 20 patients were included in the study. A total of 2915 patients met the inclusion criteria and were split into two age groups: adolescents (15 to 17 years of age) and young adults (18 to 24 years of age).

### 4.3.3 Variables

Dependent variables: Dental care outcomes were operationalized using the following DQA criteria: (a) OEV-CH-A, whether a patient had received a comprehensive or periodic oral evaluation within the year studied; this included adolescents and young adults who had at least one scheduled oral examination in a year, which included a complete exam, a recall oral exam, or an oral surgery specific exam; (b) PRV-CH-A, whether a patient received at least one preventive measure within the year studied; this included adolescents and young adults who had received either pit and fissure sealants, oral prophylaxis, or scaling; and (c) TRT-CH-A, whether a patient received at least one treatment service within the year studied; this included adolescents and young adults who had received endodontic or restorative treatment, or had a tooth extracted. These variables were operationalized as a

binary (yes or no). The cost of dental care was recorded as per the Ontario Dental Association (ODA) fee guide as well as the subsidized fee charged by the dental school.

Independent variables: Neighborhood-level SES was obtained through anonymized sociodemographic data and the first three digits of the patient's postal code, which were retrieved from the records. These were matched to data from Statistics Canada, which are stored by postal code and are readily available online (Statistics Canada Census Profile 2016). The first three characters of the postal code are referred to as the forward sortation area (FSA) code, which allows for the collection of geographic data while maintaining the confidentiality of the identity of individuals. Neighborhood-level variables included median household income, percentage of the population with less than secondary school education, percentage of the population whose language spoken at home was not an official language in Canada (i.e., neither English or French), and the percentage of the population that had lived in Canada for less than 10 years.

#### 4.3.4 Data Coding and Mapping

Coding was performed using criteria and methods that have been previously published [Podskalnyi et al., 2020]. Neighborhood-level dental care outcomes were geovisualized separately for each age group using the geographic information system software ArcGIS 10.8.1 (ESRI Canada, Toronto, ON, Canada). The data on the neighborhood-level independent variables previously mentioned were downloaded for each FSA code from the Statistics Canada database. The DQA variables for each patient were entered into the software according to their FSA code. Viable data (>20 individuals) was obtained from 17 FSA codes in the metropolitan area of London, Ontario, Canada. The entered data were used to create maps to geovisualize both neighborhood-level sociodemographic and dental care outcome variables. To facilitate visualization, maps were generated only for FSA codes (n = 14) that fell within the city limits of London, Ontario.

#### 4.3.5 Statistical Analyses

First, descriptive statistics were applied. We used the Student's t-test to assess differences in the study of sample characteristics and the cost of dental care between the two age groups. The three dental care outcomes were then compared between the two age groups

using the Mann–Whitney U test. We constructed three separate binary logistic regression models for each of the age groups to assess the association of OEV-CH-A, PRV-CH-A, and TRT-CH-A as dependent variables with neighborhood-level SES independent variables.

The sample was modelled according to FSA code using the following as covariates: median household income, percentage of the population with less than secondary school education, percentage of the population speaking a non-official language at home, and the percentage of the population that had lived in Canada for less than 10 years. The association between these neighborhood-level sociodemographic variables and individual cost of dental care was assessed using univariate linear regression models.

## 4.4 Results

A total of 2915 patients (1640 males, 1200 females, and 75 preferred not to disclose gender) from a total of 17 FSA codes in the London metropolitan area met the inclusion criteria. The mean age for patients in this sample was 19.7 years ( $SD \pm 2.9$  years) (Table 4.1). The mean cost of dental care was CAD 208 ( $SD \pm 251$ ) using the subsidized rates for the dental school and CAD 433 ( $SD \pm 526$ ) using the recommended provincial fee guide (Table 4.1). The cost of dental care was significantly greater for young adults (ODA fees CAD 512,  $SD \pm 576$ ) compared to adolescents (ODA fees CAD 194,  $SD \pm 179$ ) ( $t = -21.111$ ,  $p < 0.001$ ).

**Table 4.1: Characteristics of study sample (n = 2915)**

Age Group <sup>a</sup>	Variable	Sex	Mean	SD	t*	Sig**
15-17 years (n= 832)	Age (years)	Male	16.00	0.84	0.324	0.572
		Female	15.98	0.83		
	ODA Fees (CAD) <sup>b</sup>	Male	194.96	201.09	0.115	0.094
		Female	193.30	151.59		
	Subsidized Fees (CAD)	Male	76.48	95.80	1.600	<0.001**
		Female	66.61	46.00		
18-24 years (n= 2083)	Age (years)	Male	21.27	2.00	2.294	0.508
		Female	21.07	1.99		
	ODA Fees (CAD) <sup>b</sup>	Male	513.73	598.75	0.165	0.773
		Female	509.21	545.54		
	Subsidized Fees (CAD)	Male	253.60	280.49	0.222	0.983
		Female	250.76	260.40		

\*Calculated using the independent t test. \*\* Indicates significant difference between sexes. <sup>a</sup> Numbers do not include the 75 individuals who preferred not to disclose their gender. <sup>b</sup> Calculated based on those participants who paid for services that were billable using an ODA fee code (n = 624 for 15–17 years of age; n = 1888 for 18–24 years of age).

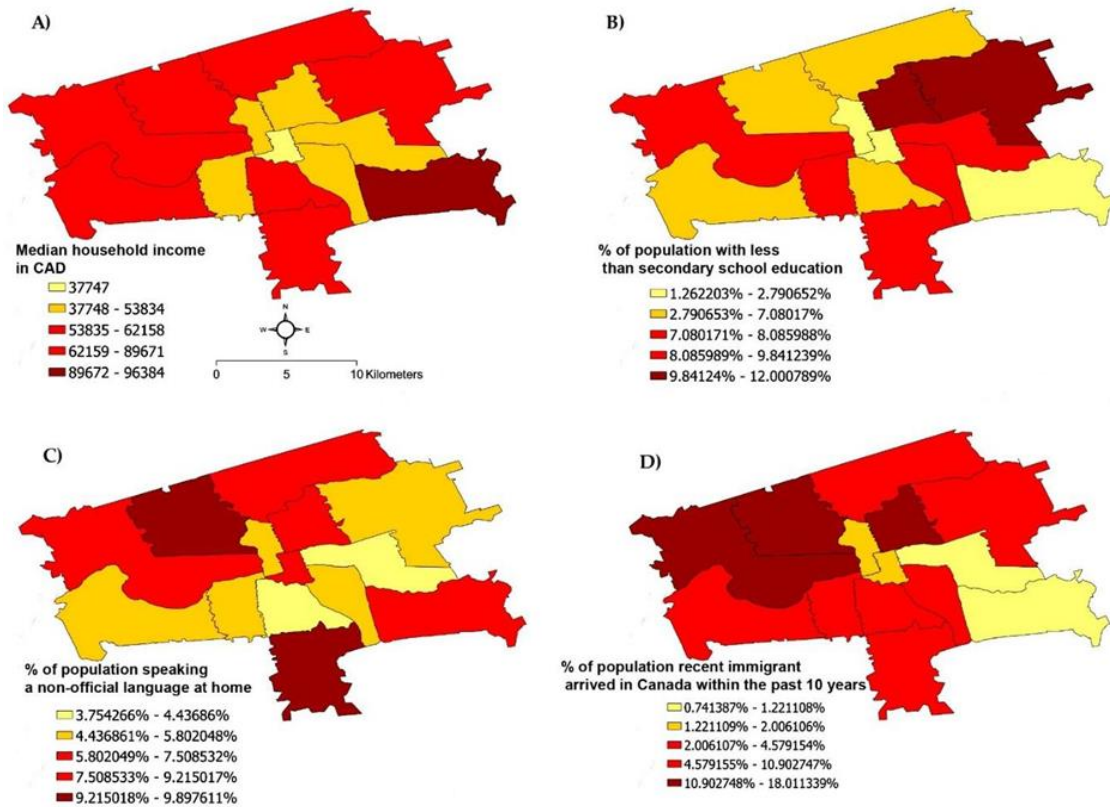
When dental care outcomes were compared between the two age groups, it was observed that, although most of the individuals in both age groups received a routine dental examination (OEV-CH-A), the proportion was significantly greater in young adults (67.9%) when compared to adolescents (56.7%). Significantly more adolescents received preventive dental services (PRV-CH-A) (30.9%) compared to young adults (18.6%). The Mann–Whitney U test found these differences to be significant for routine oral evaluation ( $p < 0.001$ ), preventive services ( $p < 0.001$ ), and treatment services ( $p < 0.001$ ) (Table 4.2).

**Table 4.2: Comparison of dental care outcomes between the two age groups**

Oral variables	Age group	DQA outcome	Observations (n = 2915)	Proportion	Sig*
Routine oral evaluation (OEV-CH-A)	15-17 years	Absent	360	43.3%	<0.001
		Present	472	56.7%	
	18-24 years	Absent	669	32.1%	
		Present	1414	67.9%	
Preventive services (PRV-CH-A)	15-17 years	Absent	575	69.1%	<0.001
		Present	257	30.9%	
	18-24 years	Absent	1695	81.4%	
		Present	388	18.6%	
Dental treatment services (TRT-CH-A)	15-17 years	Absent	302	36.3%	<0.001
		Present	530	63.7%	
	18-24 years	Absent	481	23.1%	
		Present	1602	76.9%	

\* Indicates significant difference between age groups, calculated using the Mann Whitney U Test.

The City of London has been recorded as having a wide range of sociodemographic groups (Statistics Canada Census Profile 2016). The following neighborhood-level sociodemographic variables were geovisualized and separated according to FSA code boundaries: family's median income, percentage of the population with less than secondary school education, speaking a non-official language at home, and those who had arrived in Canada within the past 10 years. The resultant maps showed distinct boundaries in terms of the four sociodemographic variables visualized, suggesting that visualization of the dental care outcome variables at an FSA level was feasible (Figure 4.1). Furthermore, the geographic distributions of the four chosen sociodemographic variables were distinct, suggesting the need to examine each as a separate variable in a regression model.

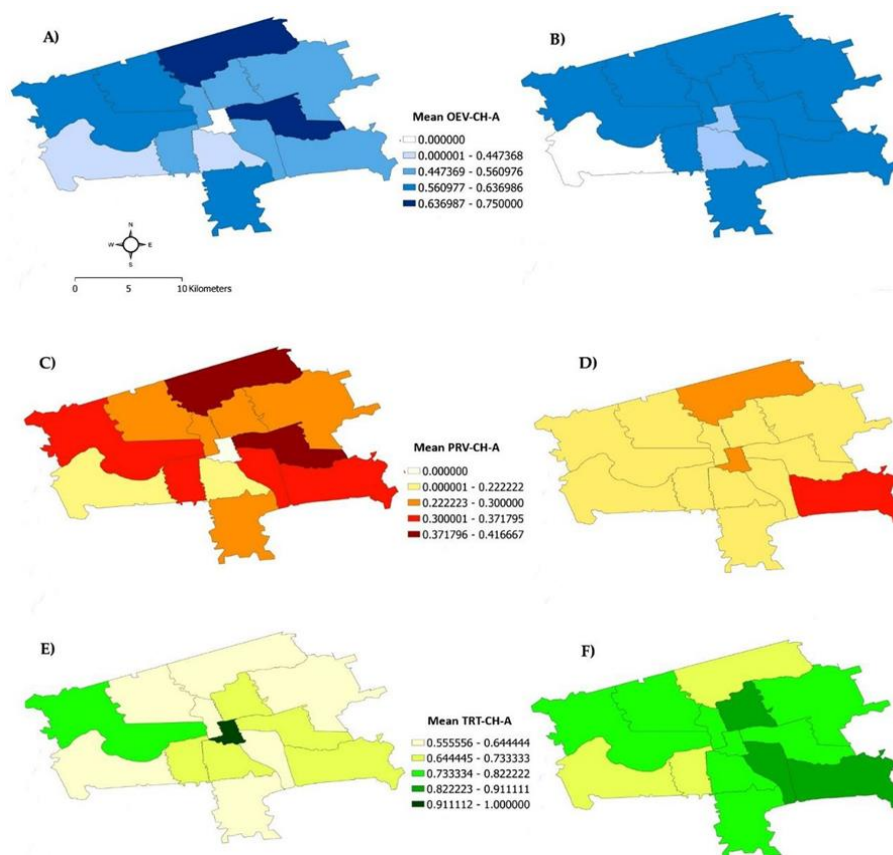


**Figure 4.1: Geographic distribution of selected sociodemographic variables with the 14 FSA codes that lie within the City of London, Ontario, Canada: (A) median household income, (B) percentage of the population with less than secondary school education, (C) percentage of the population speaking a non-official language at home, and (D) percentage of the population who are recent immigrants (arrived in Canada in the past 10 years). Maps were created using data from Statistics Canada, with each outlined area representing a single FSA code. Distance scale in A applies to all panels.**

When the dental care outcomes were geovisualized using the same 14 FSA codes, distinct patterns of care were seen. Comparing data for adolescents and young adults, it was observed that there was an overall increase in OEV-CH-A scores in young adults (Figure 4.2 A, B). However, this increase was not uniform, with some FSA codes showing an increase in OEV-CH-A (darkening in the color of the FSA code) and others showing a decrease (lightening in the color of the FSA code), suggesting potential geographic inequalities in access to routine dental care. Moreover, some areas showed a decrease in PRV-CH-A scores (lightening in the color of the FSA code) and an increase in the TRT-



CH-A score (darkening in the color of the FSA code) in young adults when compared to adolescents (Figure 4.2).



**Figure 4.2: Geographic distribution of dental care outcome variables: (A, B) routine oral evaluation (OEV-CH-A), (C, D) preventive services (PRV-CH-A), and (E, F) dental treatment services (TRT-CH-A). Data for adolescents are shown in (A, C, E). Corresponding data for young adults are shown in (B, D, F). Numerical values represent mean DQA scores on a scale from 0 to 1. Distance scale in A applies to all panels. To facilitate visualization, the maps include only FSA codes (n = 14) that fall within the city limits of London, Ontario.**

Logistic regression models showed that median household income was not significantly associated with the OEV-CHA in the population studied. However, it was observed that patients from FSA codes with a greater percentage of the population speaking a non-official language at home or recently immigrated to Canada were more likely to visit the

dental clinic of the school (Table 4.3). There was no significant association between PRV-CH-A and median household income in adolescents (OR = 1.0, 95% CI 0.8–1.1), but young adults from higher income families were more likely to receive preventive dental care than those from lower income families (OR = 1.2, 95% CI 1.0–1.3). The model also showed significant inverse associations between median neighborhood-level household income and dental treatment services in both age groups, suggesting that families with a higher median income had a lower risk of dental treatment (OR = 0.9) (Table 4.3).

**Table 4.3: Binary logistic regression models for the associations between dental care outcomes and neighborhood-level demographic variables**

	Neighborhood -Level Variables <sup>a</sup>	Oral Evaluation <sup>1</sup> (OEV-CH- A) OR (95% CI)	Preventive Services <sup>2</sup> (PRV-CH-A) OR (95% CI)	Dental Treatment Services <sup>3</sup> (TRT-CH-A) OR (95% CI)
15-17 years	Median household income	1.1 (0.9, 1.2)	1.0 (0.8, 1.1)	0.9 (0.7, 1.0)
	% of population with less than secondary school education	0.9 (0.8, 1.0)	1.1 (0.9, 1.2)	1.0 (0.8, 1.1)
	% of the population speaking a non-official language at home	1.2 (1.1, 1.4)	1.0 (0.8, 1.1)	1.0 (0.9, 1.2)
	% of population recent immigrant arrived in Canada within the past 10 years	1.2 (1.0, 1.4)	1.0 (0.9, 1.2)	1.0 (0.9, 1.2)
18-24 years	Median household income	0.9 (0.9, 1.0)	1.2 (1.0, 1.3)	0.9 (0.8, 0.9)
	% of population with less than secondary school education	0.9 (0.8, 1.0)	1.0 (0.9, 1.1)	1.1 (1.0, 1.2)
	% of the population speaking a non-official language at home	1.1 (1.0, 1.2)	1.0 (0.9, 1.2)	1.0 (0.9, 1.1)
	% of population recent immigrant arrived in Canada within the past 10 years	1.1 (1.0, 1.2)	1.0 (0.9, 1.1)	1.0 (0.9, 1.2)

<sup>a</sup> Calculated using average FSA code level data from the Statistics Canada Database, <sup>1</sup> calculated using binomial logistic regression with OEV-CH-A as dependent variable, <sup>2</sup> calculated using binomial logistic regression with PRV-CH-A as dependent variable, and <sup>3</sup> calculated using binomial logistic regression with TRT-CH-A as dependent variable.

OEV-CH-A was associated with a significant increase in the cost of care in adolescents (OR = 1.3, 95% CI 1.0–1.6), but not in young adults (OR = 0.9, 95% CI 0.8–1.0) (Table 4.4). PRV-CH-A was associated with a significant decrease in the cost of dental care in young adults (OR = 0.7, 95% CI 0.6–0.8), but not in adolescents (Table 4.4). TRT-CH-A

was associated with a significant increase in the cost of care in both adolescents (OR = 3.2, 95% CI 2.1–4.9) and young adults (OR = 18.4, 95% CI 10.8–31.6) (Table 4.4).

**Table 4.4: Binary logistic regression models for the associations between cost of care and dental care outcomes**

Dental Outcome Measure	Age group	OR (95% CI)	Sig*
Routine oral evaluation (OEV-CH-A)	15-17 years	1.3 (1.0, 1.6)	0.029**
	18-24 years	0.9 (0.8, 1.0)	0.128
Preventive services (PRV-CH-A)	15-17 years	1.1 (0.9, 1.3)	0.241
	18-24 years	0.7 (0.6, 0.8)	0.001**
Dental treatment services (TRT-CH-A)	15-17 years	3.2 (2.1, 4.9)	0.001**
	18-24 years	18.4 (10.8, 31.6)	0.001**

\* Indicates significant association between cost of care and indicated dental care outcomes.

Linear regression models with the cost of dental care as the dependent variable showed that the median household income was inversely associated with the cost of dental care, a finding that was significant in young adults ( $p < 0.001$ ), but not in adolescents ( $p = 0.161$ ) (Table 4.5). The percentage of the population with less than secondary school education was significantly associated with the cost of care among adolescents ( $p = 0.044$ ), but not young adults ( $p = 0.200$ ). There was a significant positive association between the cost of dental care and the percentage of the population that spoke a non-official language in both age groups.

**Table 4.5: The association of neighborhood-level demographic variables with cost of dental care**

Age group	Neighborhood-level variables <sup>a</sup>	B*	Sig	95% CI
15-17 years	Median household income (CAD)	-0.058	0.161	(-0.140, 0.023)
	Percentage of population with less than secondary school graduation	0.105	0.044**	(0.003, 0.207)
	Percentage of population speaking a non-official mother tongue	0.160	0.031**	(0.014, 0.306)
	Percentage of population who arrived in Canada within the past 10 years	-0.116	0.075	(-0.243, 0.012)
18-24 years	Median household income (CAD)	-0.087	<0.001**	(-0.134, -0.041)
	Percentage of population with less than secondary school graduation	0.035	0.200	(-0.018, 0.088)
	Percentage of the population speaking a non-official mother tongue	0.099	0.013**	(0.021, 0.177)
	Percentage of population who arrived in Canada within the past 10 years	-0.016	0.668	(-0.087, 0.056)

<sup>a</sup> Calculated using average FSA code level data from the Statistics Canada Database.

\* Calculated using linear regression model with cost of care as the dependent variable.

\*\* Indicates significant association.

## 4.5 Discussion

Andersen's behavioral model suggests that healthcare (including dental care) is dependent on both individual and societal elements [Andersen and Newman, 1973; Andersen, 1995]. While much of the previous work on quality of dental care has focused on the individual [Herndon et al., 2015; Ojha and Aravamudhan, 2016; Breault, 2017], it is only recently that attempts have been made to visualize societal data at a geographic level [Holmén et al., 2018; Podskalniy et al., 2020]. The transition from adolescence to adulthood is affected by a number of personal conditions, such as beliefs or attitudes, SES, and societal conditions, such as marginalization, gender, and race or ethnicity, which can facilitate or

impede the transition [Lenz, 2001; Bulgareli et al., 2018]. The present study sought to visualize the factors influencing dental care outcomes at a neighborhood level, and the differences in these outcomes between adolescents and young adults.

Our study used a cross-sectional design, assessing dental utilization through the data mining of electronic health records and billing data. This has been demonstrated to be an accurate indication of both dental caries risk and access to dental care [Herndon et al., 2015]. We utilized data from a subsidized dental clinic, to examine the impact of transitioning from adolescence to young adulthood in a population where access to dental care was available. The fact that routine oral health visits increased among young adults when compared to adolescents suggests that the access to dental care in this population did not decrease after the age of 18 years.

Access to dental care alone is not an accurate marker of the quality of dental care received, with studies showing that even when individuals have access to dental care, social and economic factors can influence the quality of care received [Tardivo et al., 2016; Bulgareli et al., 2018; Singh et al., 2019]. Our study utilized three of these measures to quantify access to routine dental care (OEV-CH-A), preventive dental services (PRV-CH-A), and treatment procedures (TRT-CH-A). The results suggest a higher OEV-CH-A among young adults when compared to adolescents, however, this must be viewed whilst keeping in mind the fact that the DQA requires a minimum of two dental visits in a year for the individual to be included in the study [Breault, 2017]. Despite having higher numbers of individuals with access to care, young adults had a significantly lower number of visits for preventive care and a significantly greater number of visits for dental treatment. This, along with the significantly greater cost of dental care among the young adults, suggests that the young adults had poorer dental care outcomes when compared to adolescents. This finding is in keeping with the pressures faced by young adults as they transition from living with their parents to living independently and making independent life choices [Kloep and Hendry, 2010]. As young adults become more independent from parental influences, they are likely to have increased responsibility for their own oral health and dental visits [Kloep and Hendry, 2010]. The fact that there was a significant change in the 18–24-year age group is

in keeping with the findings in France that resulted in creation of the M'T Dents program that extends pediatric oral health benefits up to the age of 24 years [Tardivo et al., 2016].

The average cost of dental care per patient from areas having a higher median household income was less than that of patients from areas with lower median household income. This was seen in both the 15–17-year age group as well as the 18–24-year age group, consistent with the findings of other studies showing that individuals from lower income families may end up spending more on dental care [Thompson et al., 2014; Zivkovic et al., 2020]. The findings are also in keeping with those of a similar study conducted on children below 15 years of age [Podskalniy et al., 2020], suggesting that socioeconomic determinants of healthcare affect individuals across different age groups.

It was observed that the presence of TRT-CH-A increased the cost of dental care, however, PRV-CH-A and OEV-CH-A did not. Furthermore, it was observed that, in the 18–24-year age group, there was a significant negative association between the cost of dental care and PRV-CH-A. This supports the argument made by previous studies that regular preventive dental care can reduce overall dental treatment costs [Fraihat et al., 2019; Nyamuryekung'e et al., 2019]. The findings of our study are in keeping with the idea that this relationship occurs not only at an individual level but also at a neighborhood level.

Our findings revealed that the family median income only became important after the age of 18 years. In the 18–24-year group, individuals from neighborhoods with a higher household income were significantly more likely to receive preventive services and significantly less likely to receive treatment services. This is in keeping with individual-level research among both adolescents [Kaunein et al., 2020] and adults [Sanders, 2007]. The geovisualization of these variables also showed that the changes in dental care outcomes were more pronounced in some FSA codes when compared to others. The results of this study show neighborhood-level discrepancies in dental care outcomes in both the age groups studied. This is in keeping with a previous study on children in the same population [Podskalniy et al., 2020]. However, the limited number of FSA codes did not allow for the use of more powerful geographic regression models in this study. There may be several factors that influence these geographic variations (e.g., dentist population ratios,

connectivity to the dental school, accessibility to healthcare in the neighborhood, etc.). However, there is little regional or province-wide data available on such factors, and this may be an interesting area for future research.

The results of the study need to be viewed whilst keeping in mind certain limitations. This study only examined individuals visiting the subsidized clinics of a dental school and therefore might not be representative of the entire population. Furthermore, only 17 codes were included in the study, which means that it was not possible to apply more rigorous neighborhood-level regression modelling, such as the ordinary least squares (OLS) model. There is a need for studies using a larger sample size of FSA codes and including private clinics as a source of data to explore the different socioeconomic and demographic variables documented through census data in Canada.

## 4.6 Conclusions

The results of this study show that young adults have significantly poorer preventive and treatment outcomes when compared to adolescents. Individuals from neighborhoods with lower household incomes had significantly greater costs of dental care and poorer dental care outcomes.



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## Chapter 5

### 5 Relationships among Cortisol, Perceived Stress, and Dental Caries Experience in Adolescents and Young Adults<sup>2</sup>

This chapter has been adapted from the paper titled “Relationships among Cortisol, Perceived Stress, and Dental Caries Experience in Adolescents and Young Adults” with permission of the publisher, copyright © 2024 Karger Publishers, Basel, Switzerland (see Appendix 9).

#### 5.1 Chapter Summary

Stress can impact mental and physical health, especially during adolescence and young adulthood, but the extent of its contribution to dental caries is poorly understood. The present study assessed the association between perceived stress, cortisol levels (in hair and saliva) and overall caries experience of adolescents and young adults aged 15 to 25 years. Hair and saliva samples were obtained from 93 participants free of periodontal disease. Cortisol in hair and saliva was determined using a competitive enzyme-linked immunosorbent assay. Participants completed a perceived stress questionnaire and underwent full-mouth oral examination by a calibrated examiner. Dental caries experience was based on the decayed, missing, and filled teeth (DMFT) index. Sociodemographic variables were also recorded. There were significantly higher hair cortisol levels and perceived stress scale (PSS) scores in individuals with dental caries experience (DMFT $\geq$ 1) than in those without (DMFT=0). However, there was no significant difference in salivary cortisol concentration. A binary logistic regression revealed that higher hair cortisol levels and greater scores on the perceived stress scale were associated with increased odds of having experienced dental caries. In contrast, no significant association was found between

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<sup>2</sup> A version of this chapter has been published (Abouseta N, Gomaa N, Tassi A, Elzagallai AA, Rieder MJ, Dixon SJ, Pani SC. Relationships among cortisol, perceived stress, and dental caries experience in adolescents and young adults. *Caries Research*. 2024 Apr 24: 1-10. Online ahead of print).

salivary cortisol concentration and dental caries. Using multivariable regression models, caries experience was found to be significantly associated with both hair cortisol levels and PSS scores. These associations remained statistically significant even after adjusting for sociodemographic variables. We concluded that hair cortisol levels and perceived stress have a significant association with dental caries experience, whereas salivary cortisol concentrations do not.

## 5.2 Introduction

The association of stress with the development and progression of dental caries has been previously documented [Boyce et al., 2010; Caruso et al., 2018]. Stress refers to the cognitive, emotional, and physical responses that arise when an individual experiences a discrepancy between their demands and resources [Lynch et al., 2022]. There is growing evidence that social and environmental stressors are associated with a range of diseases, including dental caries [Jain et al., 2014; Masterson and Sabbah, 2015; Sabbah et al., 2018]. Stress has been suggested to induce changes in the salivary proteome, microbiome, and salivary flow rate – each of which plays a role in oral homeostasis [Bosch et al., 2003; Sedghi et al., 2021]. Additionally, stress may affect an individual's oral hygiene practices and dietary habits, alter immune responses, increase tobacco or alcohol use, and exacerbate bruxism [Sheiham and Nicolau, 2005; Hugo et al., 2008; Peres et al., 2009; Boyce et al., 2010; Gomaa et al., 2016; Tikhonova et al., 2018; Gomaa et al., 2020], which in turn may increase the risk of dental caries. Conversely, a stress response can be triggered by various factors associated with disease [Jesenak et al., 2017]. Although it has been suggested that stress is associated with dental caries, it is important to note that not all individuals who experience stress will necessarily develop dental caries [Selwitz et al., 2007; Boyce et al., 2010; Park et al., 2022; Yang et al., 2022]. Challenges in accurately measuring stress responses have been cited as a reason for a poor understanding of the link between stress and dental caries [Gomaa et al., 2016; Tikhonova et al., 2018].

The physiological response to stress involves activation of the HPA axis, and the release of cortisol, which can have adverse effects on immune, cardiovascular, metabolic, and neural functions [Gomaa et al., 2016; Sheng et al., 2021]. Cortisol is the most widely studied marker of HPA axis activation. Hair cortisol analysis provides a retrospective

measure of cortisol levels over several months [Russell et al., 2012; Stalder and Kirschbaum, 2012; Sugaya et al., 2020]; whereas, salivary cortisol captures short-term fluctuations in response to acute stressors [Pani et al., 2013; Harris et al., 2017; Caruso et al., 2018]. It has been reported that salivary cortisol levels are increased in both periodontal disease and dental caries [Sabbah et al., 2018; Tikhonova et al., 2018]. However, it has been noted that previous studies have failed to control for the effect of gingival health while attempting to study the relationship between salivary cortisol and dental caries [Tikhonova et al., 2018].

The age range of 15 to 25 years is a period when individuals commonly experience heightened levels of stress [Siqueira et al., 2000; Lenz, 2001; Findlay, 2017]. This period is one when many individuals leave home for the first time [Lenz, 2001]. In addition, this time frame often includes the inception of self-care practices and the development of health behaviors [Yu et al., 2001; Percy, 2008; Gazzaz et al., 2021]. A recent community level study of this age group showed that access to dental treatment and preventive measures is greater in those aged 15 to 17 years when compared to those aged between 18 and 24 years [Abouseita et al., 2022].

There is little known about the correlation between salivary cortisol concentration and hair cortisol levels in individuals with minimal oral inflammation. Furthermore, the relationship between stress and dental caries in adolescents and young adults has received scant attention. Considering this gap in literature, the present study aimed to assess associations among perceived stress, cortisol levels in hair and saliva, and overall caries experience of adolescents and young adults.

## 5.3 Methods

### 5.3.1 Ethics, Consent, and Permissions

This study was approved by the Health Sciences Research Ethics Board (HSREB) at The University of Western Ontario (2021-119492). Informed consents were obtained from all adult participants (greater than 18 years of age). For minors, assents were obtained from the participants, and the parents/guardians signed the informed consent document.

### 5.3.2 Sample Power Calculation

Sample power was calculated using the G power sample size calculator (Universtat Kiel, Kiel, Germany). Calculations showed that an alpha of 0.05 with  $(1-\beta)$  of 0.95 and effect size of 0.4 would need a sample of 60. A total of 93 subjects were recruited in the study giving a post hoc power of 0.96.

### 5.3.3 Study Design

The study used a cross-sectional design and participants ranged in age between 15 and 25 years. The sample comprised medically fit individuals who received a professional dental cleaning up to two weeks before the examination. Further inclusion criteria were willingness to provide a hair sample and sufficient hair at the posterior vertex position of the head. Exclusion criteria included: gingival inflammation/bleeding during the oral examination; current chronic, autoimmune, or inflammatory disease; prolonged corticosteroid, antibiotic, or prescription medication use; current depressive or anxiety disorder; or presence of orthodontic appliances.

All subjects who consented to participate received a professional cleaning and were then scheduled for a 2-h morning appointment. Participants or parents completed a questionnaire based on the Canadian Health Measures Survey Household Questionnaire and the perceived stress scale PSS-10 survey [Cohen et al., 1983].

### 5.3.4 Collection of Hair and Analysis of Cortisol

Hair samples of approximately 20 mg (1-3 cm in length) were collected from the back of the head (posterior vertex). The hair was cut as near to the scalp as feasible using scissors and attached to a sheet of paper using Scotch® Tape. Samples were kept in envelopes at room temperature until analysis. Cortisol was extracted and analyzed according to a previously validated protocol [Greff et al., 2019] using a commercially available immunosorbent assay (11-CORHU-E01-SLV, ALPCO Diagnostics, Canada). Levels of hair cortisol were expressed as ng cortisol per g of hair.

### 5.3.5 Collection of Saliva and Analysis of Cortisol

Saliva was collected between 8:00 and 10:00 AM to minimize the effect of circadian rhythm on cortisol levels. In addition, participants were instructed to refrain from brushing their teeth, eating, or drinking for at least one hour before their appointment. Unstimulated/passive saliva was collected in a sterile polypropylene tube [Granger et al., 2007]. The samples were stored at  $-20^{\circ}\text{C}$ .

For analysis, saliva samples were thawed, vortexed, and then sedimented at 1500 RCF for 15 min. Salivary cortisol concentrations were determined according to the manufacturer's instructions, using the same immunosorbent assay kit employed for analysis of hair cortisol. The values of salivary cortisol were expressed as ng cortisol per mL of saliva.

### 5.3.6 Intraoral Examination and Recording of Dental Caries

Oral examinations were performed by a calibrated examiner (SCP) using the World Health Organization survey methods protocol [Petersen, 2008]. The examiner was calibrated on the WHO form via a repeat examination of 20 patients after a period of one week. The kappa statistic was applied to test intra-examiner reliability for decayed (including teeth that were filled but had decay) ( $\kappa=0.98$ ), missing (0.99), or filled (including tooth-colored restorations) (0.98) teeth. The absence of gingival bleeding or periodontal pockets was confirmed during the examination. The decayed, missing, and filled teeth (DMFT) index was computed from the examination form by one of the authors (NA) and used as a measure of overall caries experience.

### 5.3.7 Sociodemographic Variables and Perceived Stress

Variables related to participants' sociodemographic characteristics (age, sex, socioeconomic status, living conditions, health-related behaviors, and access to dental services (had visited a dentist or a dental hygienist in the past year) were recorded using an online questionnaire administered when obtaining consent (Qualtrics XM, Seattle, WA, USA). Total annual household income was recorded using a digital slide bar, and participants (or parents in the case of minors) were asked to drag the bar to the last reported annual family income. Household income was categorized into three groups: lower income



(less than \$29,999 CAD based on the Ontario Low-Income Workers Tax Credit), middle income (\$30,000-89,999 CAD) and higher income (more than 90,000 CAD). Participants were also asked to indicate their immigration status and the highest education attained by their parents. Educational attainment was classified as less than high school, completed high school, and having a college/ university degree or higher education. Perceived stress was measured using the 10-question perceived stress scale PSS-10 survey developed by Cohen and coworkers [Cohen et al., 1983].

### 5.3.8 Study Variables

Our outcome, caries experience, was a binary variable derived from DMFT scores. Individuals with  $DMFT \geq 1$  were categorized as having experienced dental caries and those with  $DMFT = 0$  were categorized as not having experienced dental caries. This was used as the dependent variable for statistical analyses. Independent variables included hair cortisol level, salivary cortisol concentration, and perceived stress scale score. These were used to explore potential association with caries experience, while considering different covariates.

### 5.3.9 Statistical Methods

The normality of distribution of the outcome variables was checked using the Shapiro-Wilk test and there was significant skew observed in the DMFT scores ( $SW=0.798$ ,  $p<0.001$ ), hair cortisol levels ( $SW=0.957$ ,  $p<0.005$ ), salivary cortisol concentrations ( $SW=0.905$ ,  $p<0.001$ ), and PSS scores ( $SW=0.943$ ,  $p<0.001$ ). Therefore, non-parametric statistics were used. Differences in sociodemographic and stress markers between the caries experience and no caries experience groups were assessed using the Chi-square test and Mann-Whitney U test, respectively. Correlations among salivary cortisol concentrations, hair cortisol levels, and PSS scores were assessed using Spearman's rank correlation coefficient. Cortisol values were log-transformed before regression model analysis to correct for deviations from normality. We constructed three separate binary logistic regression models to assess the extent of the association between caries experience and each of the three stress markers (hair cortisol levels, saliva cortisol concentrations, and PSS scores). Multivariable models were used to assess the extent of the association between dental caries experience and each of two stress markers (hair cortisol levels and PSS scores,

run separately), after adjustment for: 1) age and sex, 2) annual household income and parental education, and 3) dental insurance coverage and dental visits per year. All analyses were performed using the SPSS version 29 data processing software (IBM-Corp. Armonk, NY, USA).

## 5.4 Results

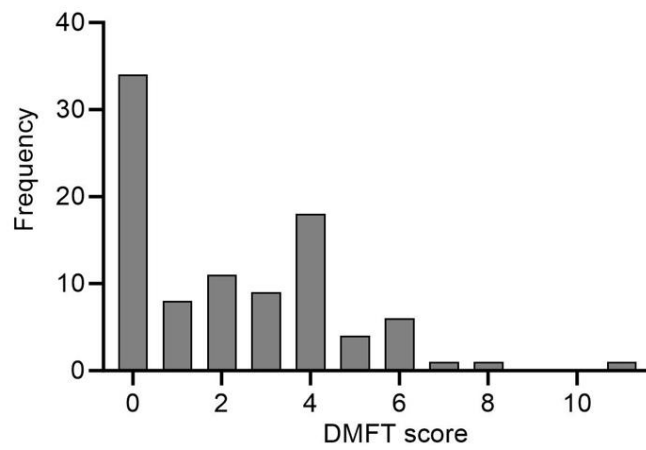
### 5.4.1 Study Sample Characteristics

Out of the 113 participants who met the inclusion criteria and consented to be examined, 20 individuals were excluded due to bleeding on probing during oral examination. Consequently, the final study sample consisted of 93 participants, (47 male and 46 female). The average age of the participants was 20 years ( $SD \pm 3$  years).

The study participants were divided into two groups – caries experience and no caries experience – to identify factors associated with dental caries. Most participants (63%) had experienced dental caries (DMFT range 1-11), while 37% had no caries experience (DMFT=0) (Figure 5.1). Table 5.1 summarizes the sociodemographic characteristics of the study population.

### 5.4.2 Presence and Absence of Dental Caries Among the Different Sociodemographic Groups

Significant differences in caries experience were observed within certain sociodemographic groups (Table 5.1). The group with incomes less than \$29,999 CAD had a greater proportion of individuals that had experienced dental caries than those in the other two income groups. Individuals with dental insurance were less likely to have dental caries than those without insurance (51% versus 71%). A substantial proportion, about 73% of the population who did not regularly visit the dentist experienced dental caries, compared to 45% of individuals who had more frequent dental visits. A greater proportion of individuals whose parents had high school education or less had experienced dental caries than those whose parents had completed at least college education. No statistically significant relationships were found between caries experience and the other variables examined in the study including, sex, age group, and immigration status.



**Figure 5.1: Histogram of the decayed, missing, and filled teeth (DMFT) scores in the overall study sample (n = 93).**

**Table 5.1: Sociodemographic profile of the study population**

Sociodemographic variable	Categories	No caries experience (N=34, 36.6%)		Caries experience (N=59, 63.4%)		P value <sup>a</sup>
		N (%)	Mean ( $\pm$ SD)	N (%)	Mean ( $\pm$ SD)	
Sex	Female	13 (28.3)		33 (71.7)		0.100
	Male	21 (44.7)		26 (55.3)		
Age			19 ( $\pm$ 3)		20 ( $\pm$ 3)	
Age Group	15-17 Years	12 (44.4)		15 (55.6)		0.471
	18-25 Years	22 (33.3)		44 (66.7)		
Household Income	$\leq$ \$29,999	9 (23.1)		30 (76.9)		0.042*
	\$30,000-89,999	12 (41.4)		17 (58.6)		
	$\geq$ \$90,000	13 (52.0)		12 (48.0)		
Dental Insurance Coverage	No	16 (28.6)		40 (71.4)		0.049*
	Yes	18 (48.6)		19 (51.4)		
Dental Visits per Year	< once a year	17 (27.4)		45 (72.6)		0.010*
	$\geq$ once a year	17 (54.8)		14 (45.2)		
Parental Education	$\leq$ High school	8 (21.1)		30 (78.9)		0.010*
	College, University or more	26 (47.3)		29 (52.7)		
Immigration Status	No	13 (31.7)		28 (68.3)		0.455
	Yes	20 (39.2)		31 (60.8)		

<sup>a</sup> Differences in caries experience within sociodemographic categories were assessed using the Chi-square test. \*Differences significant at  $p < 0.05$ .

### 5.4.3 Differences in Stress Markers According to Caries Experience

The caries experience group had significantly higher hair cortisol levels (median 211 ng/g) than the no caries experience group (141 ng/g) ( $p = 0.042$ ). In contrast, there was no significant difference in salivary cortisol concentration between the caries (13.2 ng/mL) and no caries groups (12.0 ng/mL) ( $p = 0.302$ ). Like hair cortisol levels, the caries experience group had a significantly higher mean PSS score (18.0) compared to the no caries group (13.0) ( $p = 0.026$ ) (Table 5.2, Figure 5.2). Ranges in the levels of hair cortisol were 14–404 ng/g and salivary cortisol were 4.7–32 ng/mL. PSS scores ranged from 6 to

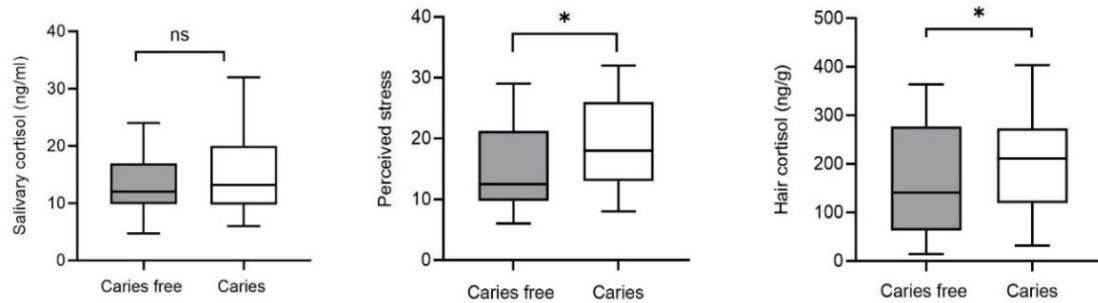
32 (scores from 0 to 13 are considered to reflect low perceived stress, 14–26 moderate stress, and 27–40 high stress [Cohen et al., 1983].

Spearman's rank correlation coefficient demonstrated a mild positive correlation between hair cortisol levels and PSS scores ( $\rho$  (rho) = 0.29,  $p$  = 0.03). However, no significant correlation was detected between hair cortisol levels and salivary cortisol concentrations ( $\rho$  (rho) = 0.11,  $p$  = 0.32), or between salivary cortisol concentrations and PSS scores ( $\rho$  (rho) = 0.07,  $p$ =0.48) (Figure 5.3).

**Table 5.2: Stress markers in the study population**

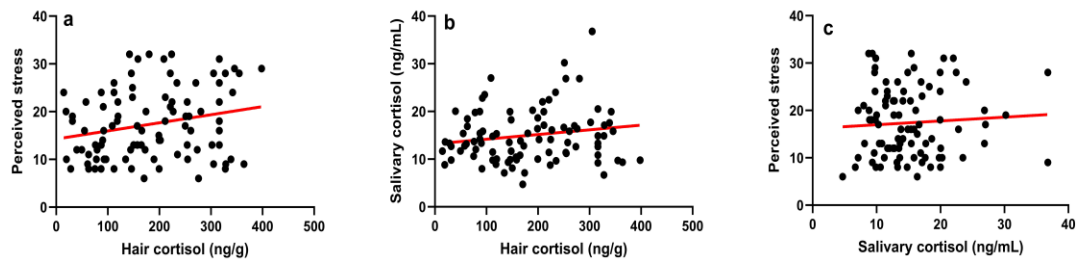
Stress marker	No caries experiences (N=34, 36.6%)		Caries experience (N=59, 63.4%)		P value <sup>a</sup>
	Median	Range	Median	Range	
Hair Cortisol (ng/g)	141	14 - 364	211	32 - 404	0.042*
Salivary Cortisol (ng/mL)	12.0	4.7 - 24.0	13.2	6.0 - 32.0	0.302
Perceived Stress Scale (PSS) Score	13	6 - 31	18	8 - 32	0.026*

<sup>a</sup> Differences in stress markers between the no caries and caries experience groups were assessed using the Mann-Whitney U test. \*Differences significant at  $p < 0.05$ .



**Figure 5.2: Stress markers in those with and without past caries experience.**

Boxes show the interquartile range, horizontal lines through the boxes represent the median, and whiskers extend to the minimum and maximum values.  $n = 34$  for the caries free group and  $n = 59$  for the caries group. Differences were assessed using the Mann-Whitney U test. \*Indicates  $p < 0.05$ .



**Figure 5.3: Spearman's rank correlation: (a) between hair cortisol levels and PSS scores; (b) between hair cortisol levels and salivary cortisol concentrations; and (c) between salivary cortisol concentrations and PSS scores. There was a positive correlation between hair cortisol levels and PSS score ( $\rho$  (rho) = 0.29,  $p = 0.03$ ). However, no significant correlation was detected between hair cortisol levels and salivary cortisol concentrations ( $\rho$  (rho) = 0.11,  $p = 0.32$ ), or between salivary cortisol concentrations and PSS scores ( $\rho$  (rho) = 0.07,  $p = 0.48$ ). The lines of best fit are indicated in red.**

#### 5.4.4 Differences in Stress Markers Among Sociodemographic Variables

Females had significantly greater hair cortisol levels when compared to males ( $p = 0.02$ ) (Table 5.3). In contrast, there was no difference in PSS scores between the two groups. There was no significant difference in hair cortisol levels or PSS scores in the two age groups (15-17 and 18-25 years of age). On the other hand, individuals with household incomes of \$29,999 or less per year had significantly higher hair cortisol levels when compared to those with higher household incomes. Household income also exhibited a significant association with perceived stress ( $p = 0.003$ ). In contrast, no statistically significant relationships were found between stress and any of the other sociodemographic variables.

**Table 5.3: Differences in stress markers among sociodemographic variables**

Sociodemographic variable	Categories	Hair cortisol level (ng/g)		PSS score	
		Mean ( $\pm$ SD)	Sig	Mean ( $\pm$ SD)	Sig
Sex <sup>a</sup>	Female	216 ( $\pm$ 85)	0.018*	16 ( $\pm$ 7.0)	0.929
	Male	170 ( $\pm$ 125)		18 ( $\pm$ 7.0)	
Age Group <sup>a</sup>	15-17 Years	177 ( $\pm$ 109)	0.413	15 ( $\pm$ 7.0)	0.091
	18-25 Years	199 ( $\pm$ 110)		18 ( $\pm$ 7.0)	
Household Income <sup>b</sup>	$\leq$ \$29,999 CAD	226 ( $\pm$ 110)	0.013*	20 ( $\pm$ 8.0)	0.003*
	\$30,000-89,999 CAD	191 ( $\pm$ 110)		13 ( $\pm$ 4.0)	
	$\geq$ \$90,000 CAD	143 ( $\pm$ 91)		18 ( $\pm$ 8.0)	
Dental Insurance Coverage <sup>a</sup>	No	197 ( $\pm$ 105)	0.527	17 ( $\pm$ 7.0)	0.637
	Yes	186 ( $\pm$ 116)		17 ( $\pm$ 8.0)	
Dental Visits per Year <sup>a</sup>	< One year	228 ( $\pm$ 130)	0.050	18 ( $\pm$ 7.0)	0.066
	$\geq$ One year	175 ( $\pm$ 93)		15 ( $\pm$ 7.0)	
Immigration Status <sup>a</sup>	No	186 ( $\pm$ 113)	0.613	18 ( $\pm$ 7.0)	0.872
	Yes	204 ( $\pm$ 105)		17 ( $\pm$ 8.0)	
Parental Education <sup>a</sup>	$\leq$ High school	162 ( $\pm$ 85)	0.051	19 ( $\pm$ 7.0)	0.082
	College, University or more	214 ( $\pm$ 119)		16 ( $\pm$ 7.0)	

<sup>a</sup> Differences in hair cortisol and PSS score between categories were assessed using the Mann-Whitney U test. <sup>b</sup> Differences in hair cortisol and PSS score among categories were assessed using the Kruskal-Wallis test. \*Differences significant at  $p < 0.05$ .

#### 5.4.5 Cortisol levels, Perceived Stress, and Dental Caries

Univariate binary logistic regression showed that individuals with greater hair cortisol levels had higher odds of having dental caries (OR=4.08, 95% CI 1.04, 15.96). A similar association was observed between the PSS score and dental caries (OR=1.65, 95% CI 1.04, 2.63). However, there was no significant association between dental caries and salivary cortisol concentration (Table 5.4). Multivariable binary logistic regression showed that the



association remained significant after adjusting for household income and parental education, and dental insurance coverage and dental visits per year (Table 5.5).

**Table 5.4: Univariate binary regression model between dental caries experience and stress markers**

Stress marker	OR (95% C.I.)	Significance*
Hair cortisol level	4.08 (1.04 - 15.06)	0.044
Salivary cortisol concentration	0.31 (0.02 - 4.23)	0.376
Score on the perceived stress scale	1.65 (1.04 - 2.63)	0.033

<sup>a</sup> Cortisol values were log-transformed to correct for deviations from normality.

<sup>b</sup> Odds ratios were calculated using binary logistic regression.

**Table 5.5: Multivariable block-wise logistic regression models examining the association between stress and dental caries experience after controlling for socioeconomic factors**

	Hair cortisol level*		Score on the perceived stress scale	
	OR (95% CI)	Sig	OR (95% CI)	Sig
Model 1	3.02 (0.71, 12.88)	0.135	1.66 (1.03, 2.66)	0.036
Model 2	5.04 (1.03, 24.65)	0.046	2.02 (1.14, 3.58)	0.015
Model 3	5.62 (1.02, 31.07)	0.048	2/09 (1.15, 3.80)	0.016

The association between caries experience and hair cortisol or perceived stress:

Model 1: Adjusted for age and sex.

Model 2: Additionally adjusted for household income and parental education.

Model 3: Additionally adjusted for dental insurance coverage and dental visits per year.

\*Cortisol values were log-transformed to correct for deviation from normality.

## 5.5 Discussion

It has been suggested that psychosocial and biological factors play an integral part in the oral health of adolescents and young adults [Baker et al., 2010; Silva et al., 2018]. These factors include social relationships, academic and financial concerns, work demands, and access to healthcare [Findlay, 2017; Condon, 2018; Koga et al., 2020; Gazzaz et al., 2021]. Therefore, factors associated with the oral health of adolescents and young adults may be distinct from those of children and adults. The present study examined relationships among dental caries, stress, and cortisol in individuals 15 to 25 years of age.

Previous studies have reported an association between perceived stress and caries experience [Jain et al., 2014]. Our findings are in keeping with these observations, with perceived stress scale scores being positively associated with caries experience. However, not all participants with low stress scores had no caries experience and there were some individuals having no caries experience with high stress scores. These observations suggest that the association between stress scores and caries experience is not causative and are consistent with the multifactorial etiology of dental caries.

The potential of cortisol as a marker for dental caries has been investigated in the past with different outcomes [Tikhonova et al., 2018]. Both hair and salivary cortisol are proven markers of stress, with literature suggesting that, while hair cortisol is a marker for long term stress, salivary cortisol is more indicative of short- term stress and fear [Kambalimath et al., 2010; Cruickshank et al., 2021]. Although some studies on early childhood caries have shown an association between salivary cortisol and dental caries, others have shown no association [Kambalimath et al., 2010; Yfanti et al., 2014; Tikhonova et al., 2018]. Similarly, the association between stress and hair cortisol is well established; however, there is little in the literature on the association between hair cortisol and dental caries. In a case-control study of preschool children with severe early childhood caries, there was no significant association between hair cortisol and caries [Angelhoff et al., 2023]. To the best of our knowledge, ours is the first study to report a positive association between hair cortisol levels and dental caries experience.

There are several potential mechanisms that may underlie the association between dental caries experience and chronic stress, measured as hair cortisol levels. Behavioral changes and impaired coping mechanisms may result from stress [Aldwin, 2011]. In this regard, stress may lead to: poor oral hygiene practices, such as irregular brushing and flossing; habits such as smoking and excessive alcohol consumption; and ingestion of sugary snacks or drinks, all of which are associated with dental caries [Tikhonova et al., 2018]. Additionally, bruxism – a prevalent parafunctional habit linked to chronic stress [Wieckiewicz et al., 2014] – has been associated with dental caries [Topaloglu-Ak et al., 2022].

Besides behavioral changes, it is possible that stress modulates pathophysiological mechanisms that in turn predispose an individual to dental caries. For example, stress can affect the immune system, compromising host resistance to cariogenic bacteria [Lawrence et al., 2005; Gomaa et al., 2016]. In this regard, stress can disrupt the production of secretory immunoglobulin A, which is essential for defending against pathogens and maintaining oral microflora balance [Lawrence et al., 2005]. Saliva characteristics are also altered by stress through the effects of stress on the autonomic nervous system, which in turn influences salivary gland function [Tikhonova et al., 2018]. These functions include salivary flow rate, composition, and the ability to induce microbial adherence [Bosch et al., 2003].

On the other hand, it is also conceivable that suffering from dental caries leads to stress. For example, it has been suggested that chronic pain from dental caries causes discomfort, hindering daily activities and disrupting sleep, thus contributing to chronic stress [Bosch et al., 2003; Tikhonova et al., 2018]. Moreover, financial concerns related to dental treatment and fear of dental procedures may contribute to stress, particularly among lower socioeconomic groups [Boyce et al., 2010; Tikhonova et al., 2018]. In summary, the link between dental caries experience and chronic stress remains ambiguous but may involve multiple factors. Further research is clearly needed to elucidate the mechanisms underlying the association between stress and caries.

Our study found no correlation between levels of hair and salivary cortisol; however, we did find a positive correlation between hair cortisol levels and perceived stress scores. Literature shows that the association between hair cortisol level and salivary cortisol concentration is inconsistent, with discrepancies being attributed to differences in the temporal characteristics of the measurements and the functional activity of the hypothalamic-pituitary-adrenal axis [Zhang et al., 2018; Sugaya et al., 2020].

The association between socioeconomic factors and dental caries is well established in literature [Singh et al., 2019; Fang et al., 2021; Sääksjärvi et al., 2021]. It was therefore important to address whether these findings were a source of bias. Our results suggest that the association between stress and dental caries was valid even when adjusted for socioeconomic status of the family, parental education, dental insurance coverage, and dental visits per year.

The results of this study must be viewed keeping in mind certain limitations. The study relied on relatively small sample size. Moreover, we used a convenience sample to facilitate data collection, and this does not accurately reflect the population as a whole. Although the use of a cross-sectional study design allowed us to test the association between stress, cortisol and dental caries in a relatively small sample, the design did not allow us to predict caries risk.

Another limitation was that caries experience was dichotomized, thereby negating the possible effect of caries severity. Even though our findings show greater dental caries experience and stress among individuals who are socioeconomically disadvantaged, the size of the current sample did not allow for detailed exploration of the impact of sociodemographic variables. The questionnaire provided self-reported data, which may be subject to biases and relies on individuals to accurately report their own thoughts, behaviors, or experiences. Other oral health risk factors were not investigated; for example, cariogenic bacteria and the level of dietary carbohydrates were unmeasured in our study. However, as both hair cortisol and oral health data are being collected at the national level by the Canadian Health Measures Survey (CHMS), future studies could use secondary data from this database to further explore sociodemographic outcomes.

## 5.6 Conclusions

Within the limitations of this study, we can state that hair cortisol levels – which reflect chronic stress – have a significant association with dental caries, whereas salivary cortisol concentrations do not. It is conceivable that stress can lead to unfavorable oral health behaviors resulting in dental caries.

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## Chapter 6

### 6 Profiling mRNA Encoding Glucocorticoid Receptor $\alpha$ in Saliva: Relationship to Hair Cortisol Levels in Individuals Aged 15-25 Years<sup>3</sup>

#### 6.1 Chapter Summary

We assessed levels of mRNA encoding two glucocorticoid receptor (GR) isoforms (GR $\alpha$  and GR $\beta$ ) in saliva and examined their relationship with hair cortisol levels and dental caries experience. Adolescents and young adults were assessed for dental caries experience, and hair cortisol was measured by ELISA. RNA was extracted from whole saliva using TRIzol, followed by quantitative real-time PCR analysis of GR $\alpha$ , GR $\beta$ , and glyceraldehyde 3-phosphate dehydrogenase (GAPDH). GR $\beta$  mRNA was not detectable in most samples, whereas GR $\alpha$  mRNA was observed in all samples. There were significantly lower levels of GR $\alpha$  mRNA in individuals with elevated hair cortisol levels than in those with normal cortisol levels. Levels of GR $\alpha$  mRNA did not differ significantly in individuals with dental caries experience compared to individuals with no caries experience. In conclusion, we identified and quantified mRNA encoding GR $\alpha$  in saliva. Its levels were inversely associated with hair cortisol (a marker of chronic stress). Although caries experience was associated with hair cortisol levels, there was no significant association between GR $\alpha$  levels and caries experience. Chronic stress has been proposed to be associated with reduced expression of GR $\alpha$  and this association appears to hold for GR $\alpha$  mRNA levels in saliva.

#### 6.2 Introduction

Dental caries is a multifactorial disease involving complex interactions between environmental factors, microbial flora, and host-related factors. Of the different host-

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<sup>3</sup> A version of this chapter has been submitted for publication (Abouseta N, Gomaa N, Tassi A, Dixon SJ, Singh K, Pani SC. Profiling mRNA encoding glucocorticoid receptor  $\alpha$  in saliva: Relationship to hair cortisol levels in individuals aged 15-25 years).

related factors influencing the development and progression of dental caries, the role of stress pathways mediated by the glucocorticoid hormone cortisol has received some attention in literature. The hypothalamic-pituitary-adrenal (HPA) axis is the primary regulator of an individual's response to stress. It begins with the secretion of corticotropin-releasing hormone from the parvocellular neurons of the hypothalamus, signaling the pituitary gland to release adrenocorticotropic hormone (ACTH). ACTH then stimulates the adrenal cortex to release cortisol. Cortisol, a glucocorticoid, mediates its actions through glucocorticoid receptor (GR)  $\alpha$  [Smith and Vale, 2006], influencing gene expression and modulating processes such as energy metabolism, immune function, and inflammatory responses. As cortisol levels are elevated, a negative feedback loop is activated to prevent excessive activation of the stress response. Simultaneously, this process triggers a downregulation of GR $\alpha$  expression at the cellular level [Bamberger et al., 1996; Vassiliou et al., 2019]. In turn, this downregulation can increase susceptibility to diseases [Nikkheslat et al., 2015; Keller et al., 2017].

Disruptions in the body's stress response system can affect saliva composition, the oral microbiome, blood sugar regulation, and coping mechanisms [Bosch et al., 1996; Duran-Pinedo et al., 2018]. Previous research has indicated that inadequate coping strategies are associated with the stress-health relationship and can amplify the relationships among oral diseases, systemic diseases, and stress [Heidari Pahlavian et al., 2010; Gomaa et al., 2019]. This could include an increased consumption of sugary foods and beverages, smoking, alcohol consumption, and less frequent tooth-brushing [Slopen et al., 2013; Pohjola et al., 2021]. Cortisol is the product of the HPA pathway and has been extensively studied as a marker of stress [Tsigos and Chrousos, 2002]. GR $\alpha$  respond to circulating glucocorticoids and are responsible for attenuating elevated glucocorticoid levels and returning the HPA-axis to homeostatic levels [Morimoto et al., 1996; De Kloet et al., 1998]. In humans, two GR mRNA splice variants leading to two protein isoforms (GR $\alpha$  and GR $\beta$ ) may contribute to differences in glucocorticoid receptor responsiveness [Smith and Vale, 2006]. The predominant and functionally active isoform is GR $\alpha$ , a 777-amino acid protein. On the other hand, GR $\beta$  is a shorter isoform consisting of 742 amino acids [Revollo and Cidlowski, 2009]. GR $\beta$  does not bind glucocorticoids and may function as a dominant negative inhibitor of the effects of cortisol [Vassiliou et al., 2019].

Chronic stress and HPA hyperactivity have been proposed to be associated with reduced GR gene expression and activation [Juruena et al., 2004; Gądek-Michalska et al., 2013]. These alterations disrupt the HPA axis, ultimately influencing the response to stress and increasing the risk of disease [Miller et al., 2002; Smith and Vale, 2006; Panagiotou et al., 2021]. Therefore, assessing GR expression, in addition to cortisol levels, provides important insight into the overall state of HPA axis activation and responsiveness to stressors [Vasiliou et al., 2016; Vassiliou et al., 2019]. For these reasons, GR expression has been proposed as a measure of HPA axis activation [DeRijk et al., 2002; Juruena et al., 2004].

We previously studied a population of adolescents and young adults (15 to 25 years of age). We found that individuals with caries experience had higher levels of hair cortisol and perceived stress than those with no caries experience. In contrast, there was no significant difference in salivary cortisol concentration. The associations between high hair cortisol levels and perceived stress with dental caries experience remained statistically significant even after adjusting for confounding sociodemographic variables.

The primary objective of the present study was to measure GR transcript levels in the whole saliva. The study investigated the relationship of GR transcript levels to hair cortisol levels – an indicator of chronic stress. We also examined the relationship of GR transcript levels to dental caries experience. The study specifically aimed to: 1. Compare GR transcript levels between individuals with and without elevated hair cortisol levels. 2. Compare GR transcript levels between individuals with and without dental caries experience. 3. Compare GR transcript levels among the following groups of individuals: (a) with dental caries experience and with normal hair cortisol levels; (b) without dental caries experience and with normal hair cortisol levels; (c) with dental caries experience and with elevated hair cortisol levels; and (d) without dental caries experience and with elevated hair cortisol levels.

## 6.3 Materials and Methods

### 6.3.1 Ethics, Consent, and Permissions

The study was conducted in accordance with the guidelines of the Declaration of Helsinki.

Ethics approval was obtained from the Health Science Ethics Review Board of the University of Western Ontario (ID-119492, 20 September 2021). Informed consent was obtained from all adult participants (above 18 years of age). For minors, the participant provided verbal consent, and the informed consent document was signed by their parents.

### 6.3.2 Study Design

The study included subjects (n=93), aged between 15-25 years (46 males and 47 females) recruited from the dental clinics of the Schulich School of Medicine & Dentistry at the University of Western Ontario. These were the same subjects that had been included in our previous study on the “Relationships among Cortisol, Perceived Stress, and Dental Caries Experience in Adolescents and Young Adults”. All participants received professional dental cleaning two weeks prior to examination. The criteria for exclusion included bleeding on probing during the oral examination; having chronic, autoimmune, or inflammatory diseases; being on long-term corticosteroids, antibiotics, or prescription medications; having current diagnoses of depression or anxiety disorders; and using orthodontic devices.

### 6.3.3 Measurement of Caries Experience

Oral health status was recorded using the World Health Organization survey methods protocol [Petersen, 2008]. We calculated the number of decayed, missing and filled permanent teeth (DMFT index). Individuals with a DMFT score of 1 or higher were categorized as having experienced dental caries, while all others were categorized as caries-free [Abouseta et al., 2024].

### 6.3.4 Measurement of Hair Cortisol Levels

Hair cortisol was measured using a commercially available ELISA kit as described previously [Abouseta et al., 2024]. Hair cortisol levels were treated as an independent variable. Individuals with hair cortisol levels over the reference range for normal (17.7–153.2 ng/g) [Sauvé et al., 2007] were classified as having high cortisol levels, while all other individuals were categorized as having a normal cortisol level.

### 6.3.5 Extraction of mRNA from Saliva

Participants refrained from eating, drinking, or performing oral hygiene procedures for at least an hour before sample collection. Whole saliva was collected using a passive drool technique between 9 and 11 AM [Granger et al., 2007]. Saliva samples were divided into 1 mL aliquots in 2 mL sterile tubes and centrifuged at 16,000 RCF at 4°C for 20 min. Saliva pellets were homogenized in 1 mL TRIzol reagent (Invitrogen) (Ambion, Cat# 15596018, Lot# 318303) and pipetted several times, followed by vortex mixing for 20 s [Chomczynski and Mackey, 1995]. Phase separation was performed by adding 200 µL chloroform and shaking vigorously by hand for 15 s followed by 3-min incubation at room temperature (RT) before centrifuging at 12,000 RCF at 4°C for 15 min. The clear, upper RNA phase (about 600 µL) was transferred to a new RNase-free tube. RNA precipitation was performed by adding 500 µL isopropyl alcohol and the tube was inverted 5 times before incubating for 10 min at RT. Then the tube was centrifuged at 12,000 RCF at 4°C for 10 min to pellet the RNA. The supernatant was removed, and the pellet was washed with 1 mL of 75% ethanol. Then the tube was inverted 5-times prior to being centrifuged at 7,500 RCF at 4°C for 5 min and then ethanol was removed; this step was repeated once. The pellet was allowed to be partially air-dried and was subsequently dissolved in 25 µL diethyl pyrocarbonate (DEPC) water. To facilitate dissolution of the RNA, the tube was vortexed and incubated at 65°C for 3 min, followed by 2-3 min incubation on ice. The total RNA concentration was measured by NanoDrop-1000 (Nanodrop, Wilmington, DE, USA), and stored at -80°C. RNA concentrations in the whole saliva samples ranged between 158 and 1379 ng/µL ( $578 \pm 247$  ng/µL, mean  $\pm$  SD). The ratio of absorbance at 260 and 280 nm ( $A_{260}/A_{280}$ ) was  $2.1 \pm 0.13$  (mean  $\pm$  SD), indicating acceptable RNA purity. The  $A_{260}/A_{230}$  absorbance ratio was  $2.0 \pm 0.16$  (mean  $\pm$  SD) consistent with minimal contamination by salts, phenol, or peptides.

### 6.3.6 cDNA Synthesis and Real-Time Quantitative PCR

Quantitative real-time PCR (qPCR) was used to amplify and quantify target gene mRNA in a sample. One µg of total RNA from each sample was used to synthesize complementary DNA (cDNA) using the QuantiTect Reverse Transcription Kit (Qiagen, Canada), followed by qPCR using Thermal Cycler Real-Time PCR machine (Eppendorf).

The qPCR reaction was performed with SYBR Select Master Mix (Applied Biosystems, Cat# 4472903, Thermo Fisher Science, Canada) on the Quantstudio 3™ (Thermo Fisher, Waltham, MA, USA). The primers for glyceraldehyde 3-phosphate dehydrogenase (GAPDH, endogenous reference gene) [Singh et al., 2013] and the target GR $\alpha$  and GR $\beta$  genes [Panagiotou et al., 2021] are listed in Table 6.1. Quantitative real-time PCR was performed in 384-well plates in duplicate using QuantStudio®3 Real-Time PCR Instrument (Applied Biosystems). Each The qPCR conditions were 95°C for 2 min followed by 40 cycles at 95°C for 15 s and 60°C for 1 min. Amplification of the single PCR product was confirmed by monitoring the dissociation curve. The amplification curve was automatically adjusted to establish a suitable baseline and threshold cycle.

**Table 6.1: Oligonucleotide primers for real-time PCR**

Genes	Sequences
<i>GAPDH-F</i>	5'-CACCAGGGCTGCTTTTAACTCTGGTA-3'
<i>GAPDH-R</i>	5'-CCTTGACGGTGCCATGGAATTTGC-3'
<i>GR<math>\alpha</math>-F</i>	5'-TGACTCTACCCTGCATGTACGACCA-3'
<i>GR<math>\alpha</math>-R</i>	5'-TCAGCTAACATCTCGGGGAATTCAA-3'
<i>GR<math>\beta</math>-F</i>	5'-GAAGGAACTCCAGCCAGAA-3'
<i>GR<math>\beta</math>-R</i>	5'-CCACATAACATTTTCATGCATAGA-3'

The Ct levels, which indicate the cycle threshold of a PCR reaction, are inversely proportional to the amount of target RNA in the sample. The Ct value was calculated as the average from the duplicate test samples for each participant. Out of the initial 93 participants screened, 78 were ultimately included in the study after omitting samples in which the difference between the Ct values for duplicate wells was more than one (n = 14 participants) or which were outliers (n = 1 participant, more than 3 SD from the mean). The difference in the Ct values between the genes of interest and the GAPDH gene was used for subsequent investigation and defined as delta Ct ( $\Delta$ Ct). The average Ct values for



GAPDH and GR $\alpha$  were (23.3 and 30.2, respectively). We computed the reciprocal of  $\Delta$ Ct and used it as a measure of mRNA levels.

### 6.3.7 Statistical Analyses

The normality of the distribution of  $1/\Delta$ Ct values was checked using the Shapiro-Wilk test. It was normally distributed (SW = 0.306,  $p = 0.321$ ). Hair cortisol levels were also normally distributed (SW = 0.33,  $p = 0.13$ ). Therefore, parametric statistics were used. Independent sample t-tests were used to compare differences between the mean GR $\alpha$  value ( $1/\Delta$ Ct) of the caries and caries-free groups, and the high and normal hair cortisol groups, as well as differences in hair cortisol levels between the caries and caries-free groups. One-way analysis of variance (ANOVA) was used to compare differences in GR $\alpha$  mRNA levels and hair cortisol levels among groups, followed by a least significant difference (LSD) post hoc-test to compare differences between individual groups. A linear regression model was constructed to investigate the relationship among cortisol levels in hair, dental caries experience, and levels of GR $\alpha$  mRNA. The model used GR $\alpha$  values ( $1/\Delta$ Ct) as the dependent variable and hair cortisol and dental caries experience as independent variables. All tests were performed with a level of significance of  $p < 0.05$ . Pearson's correlation and Spearman's rank correlation were conducted between levels of GR $\alpha$  mRNA, and hair cortisol levels and dental caries experience, respectively. Spearman's rank correlation was also conducted between hair cortisol levels and dental caries experience. All statistical analyses were performed using the SPSS ver.26 data processing software (IBM corp., Armonk, NY USA).

## 6.4 Results

### 6.4.1 Participant Characteristics

There were 78 participants – 37 males and 41 females (Table 2). The average age of the participants was  $20 \pm 3$  years (mean  $\pm$  SD). The mean hair cortisol level was  $177.1 \pm 65.7$  ng/g. Hair cortisol levels were normally distributed (skew=0.306). Among the participants, 65% had experienced dental caries, while 35% had not experienced dental caries. GR $\beta$  mRNA was not detected due to very low expression in most of the samples that were examined. Conversely, GR $\alpha$  mRNA was observed in all samples analyzed.

**Table 6.2: Characteristics of participants**

		N (%)	Mean	Standard Deviation
Sex	Female	41 (52.6)		
	Male	37 (47.4)		
Age (years)			20.0	3.0
Hair Cortisol (ng/g)			177.1	65.7
Caries Experience	Dental caries	51 (66.7)		
	Caries-free	26 (33.3)		

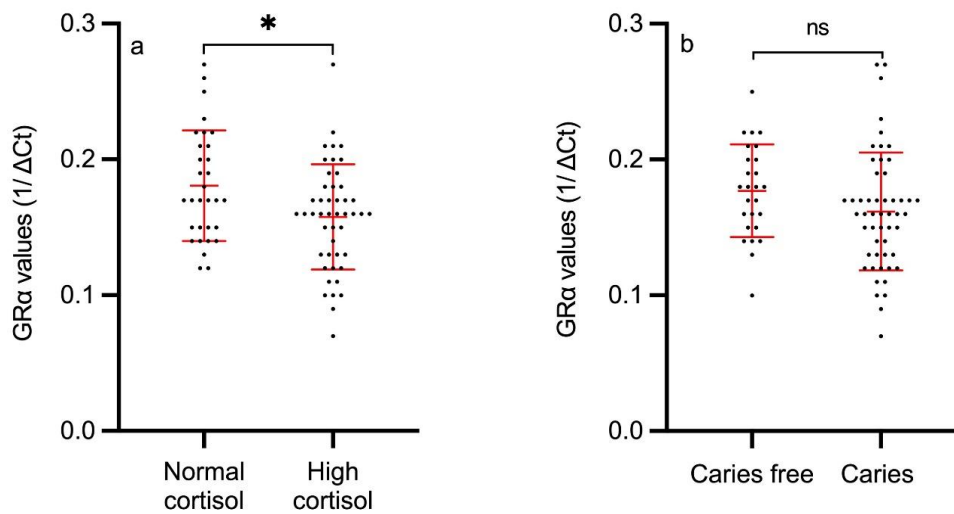
#### 6.4.2 Comparison of GR $\alpha$ mRNA Levels in Designated Groups

The independent sample t-test did not show a significant difference for GR $\alpha$  mRNA levels ( $1/\Delta\text{Ct}$ ) between sexes ( $p = 0.389$ ) (Table 6.3). On the other hand, individuals with normal hair cortisol showed significantly higher GR $\alpha$  mRNA levels ( $0.18 \pm 0.04$ , mean  $\pm$  SD) than those with elevated hair cortisol levels ( $0.16 \pm 0.04$ ) ( $p = 0.007$ ) (Figure 6.1a). In contrast, there was no significant difference in GR $\alpha$  mRNA levels between the participants with caries experience ( $0.16 \pm 0.04$ ) and those with no caries experience ( $0.18 \pm 0.03$ ) ( $p = 0.061$ ) (Figure 6.1b). On the other hand, there was a significant difference in hair cortisol levels between participants with caries experience ( $190.3 \pm 74.6$  ng/g) and those with no caries experience ( $148.2 \pm 30.6$  ng/g) ( $p = 0.004$ ).

**Table 6.3: Levels of mRNA encoding GR $\alpha$** 

			GR $\alpha$ value (1/ $\Delta$ Ct)			
		N	Mean $\pm$ SD	Range	95% CI	<i>p</i> value*
Sex	Female	41	0.17 $\pm$ 0.04	0.09 - 0.27	-0.02 to 0.02	0.387
	Male	37	0.17 $\pm$ 0.04	0.07 - 0.25		
Hair cortisol level	Normal	31	0.18 $\pm$ 0.04	0.12 - 0.27	0.01 to 0.04	0.007
	High	47	0.16 $\pm$ 0.04	0.07 - 0.27		
Caries experience	Caries-free	26	0.18 $\pm$ 0.03	0.10 - 0.25	0.00 to 0.04	0.061
	Caries	52	0.16 $\pm$ 0.04	0.07 - 0.27		

\*Calculated using the one-tailed independent sample t-test



**Figure 6.1: (a) GR $\alpha$  values (1/  $\Delta$ Ct) for individuals with normal and high levels of hair cortisol. Data means  $\pm$  SD, n = 31 participants for the normal cortisol group and n = 47 participants for the high cortisol group. \*\* t-test showed significant difference (p<0.01). (b) GR $\alpha$  values (1/ $\Delta$ Ct) for caries-free individuals and those with caries experience. Data means  $\pm$  SD, n = 26 participants for the caries-free group and n = 52 participants for the caries group. \* The independent sample t-test showed no significant difference (p < 0.061).**

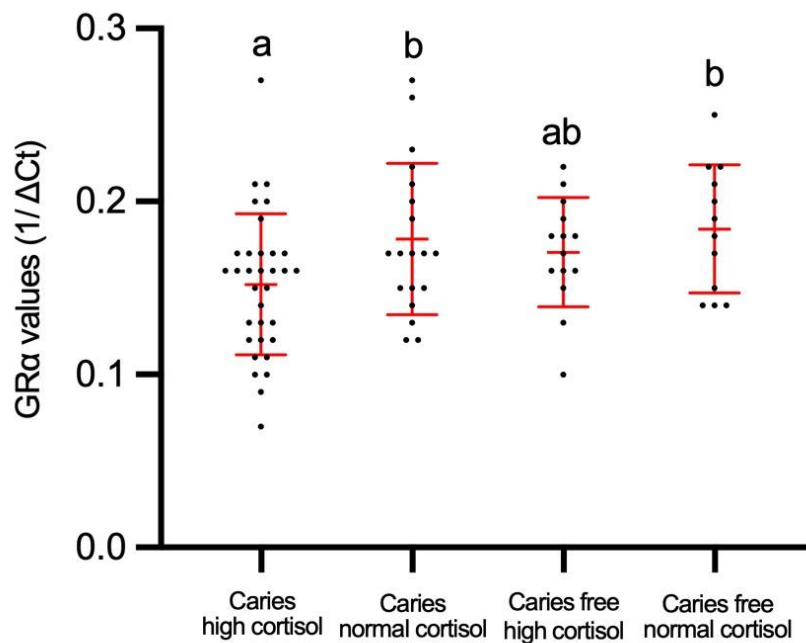
#### 6.4.3 Differences in GR $\alpha$ mRNA Levels and Hair Cortisol Among Four Groups: Caries or Caries-Free and Normal or High Cortisol

One way ANOVA showed a significant difference in GR $\alpha$  (1/ $\Delta$ Ct) among the groups being examined (dental caries experience/high cortisol, dental caries experience/normal cortisol, caries-free/high cortisol, and caries-free/normal cortisol) (p = 0.041).

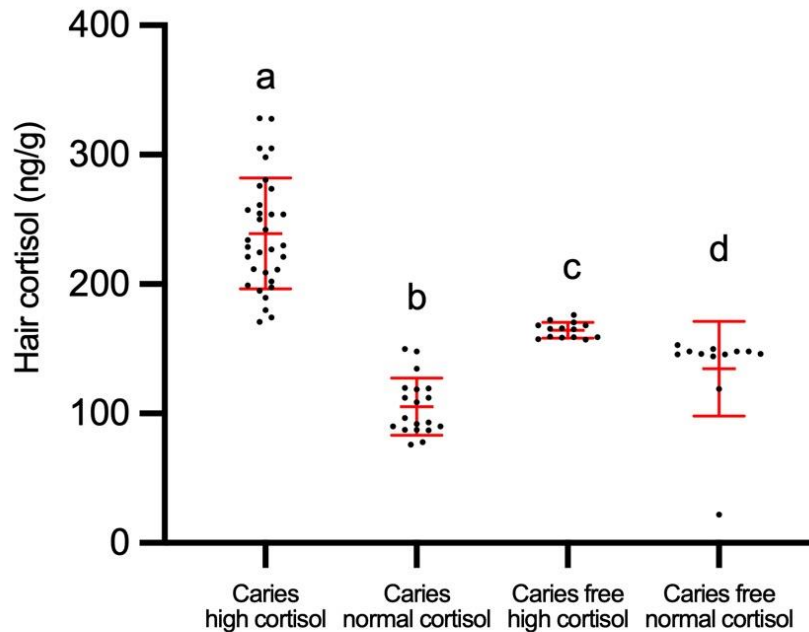
The LSD post hoc test showed that the caries/high cortisol group and caries/normal cortisol group were significantly different from each other. Additionally, the caries/high cortisol group and caries-free/normal cortisol group were significantly different from each other (Figure 6.2). In contrast, there was no significant difference in GR $\alpha$  mRNA levels in the

high cortisol group between individuals with and without dental caries experience (Figure 6.2).

Additionally, one way ANOVA on hair cortisol levels for the 4 groups revealed a significant difference ( $p = 0.001$ ). The LSD post hoc test showed that all groups were significantly different from each other (Figure 6.3).



**Figure 6.2: Differences in GR $\alpha$  values ( $1/\Delta Ct$ ) between the four groups: dental caries experience & high cortisol, dental caries experience & normal cortisol, caries-free & high cortisol, and caries-free & normal cortisol. Data means  $\pm$  SD. For caries & high cortisol,  $n = 33$  participants; for caries & normal cortisol,  $n = 19$  participants; for caries-free & high cortisol,  $n = 14$  participants; and for caries-free & normal cortisol,  $n = 12$  participants. Data were analysed by one-way ANOVA and LSD post hoc test. Dot plots labelled with the same lowercase letter are not significantly different from each other.**



**Figure 6.3: Differences in hair cortisol levels between the four groups: dental caries experience & high cortisol, dental caries experience & normal cortisol, caries-free & high cortisol, and caries-free & normal cortisol. Data means  $\pm$  SD. For caries & high cortisol, n = 33 participants; for caries & normal cortisol, n = 19 participants; for caries-free & high cortisol, n = 14 participants; and for caries-free & normal cortisol, n = 12 participants. Data were analysed by one-way ANOVA and LSD post hoc test. Dot plots labelled with different lowercase letters are significantly different from each other.**

#### 6.4.4 The association Between Hair Cortisol, Dental Caries Experience and GR $\alpha$ mRNA Levels

A linear regression model was constructed between GR $\alpha$  as a dependent variable and hair cortisol and dental caries experience as independent variables. There was a significant association among the three variables ( $p < 0.001$ ). Hair cortisol was found to be negatively associated with GR $\alpha$  ( $p < 0.001$ ). In contrast, there was no significant association between dental caries experience and GR  $\alpha$  levels ( $p = 0.397$ ) (Table 6.4).

Pearson correlation analysis demonstrated a significant negative correlation between GR $\alpha$  mRNA levels and hair cortisol levels (Figure 6.4,  $r = -0.476$ ,  $p < 0.001$ ). The scatter plot illustrates an improvement in the goodness of fit after the cut off point for normal versus high hair cortisol (153 ng/g). The correlation was significant in the high cortisol group ( $r = -0.888$ ,  $p < 0.001$ ) but not in the normal cortisol group ( $r = -0.097$ ,  $p = 0.605$ ). The Spearman's rank correlation showed no significant correlation between GR $\alpha$  mRNA levels and dental caries experience ( $\rho$  (rho) =  $-0.212$ ,  $p = 0.062$ ). In contrast, the Spearman's rank correlation showed a significant positive correlation between hair cortisol levels and dental caries experience ( $\rho$  (rho) =  $0.277$ ,  $p = 0.014$ ).

**Table 6.4: Linear regression model to assess the relationships between GR $\alpha$  and hair cortisol, and GR $\alpha$  and caries experience**

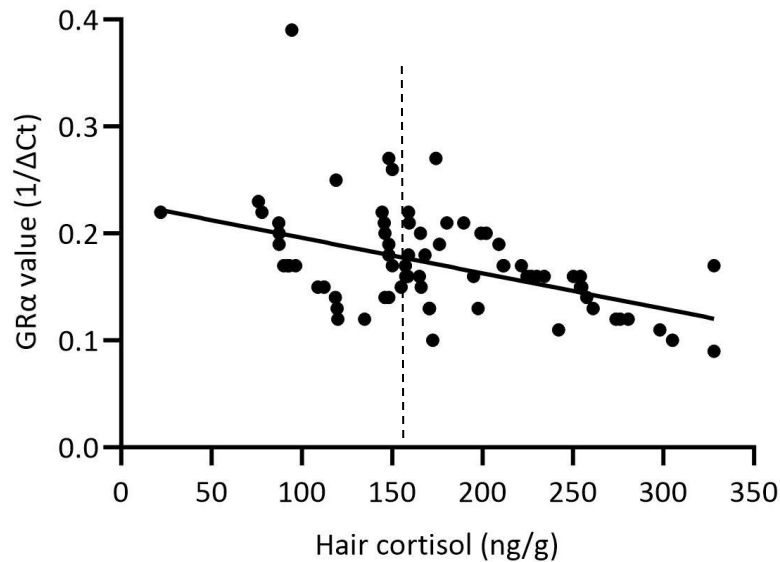
Variable	Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95 % Confidence Interval for B	
	B	Standard Error	Beta			Lower Bound	Upper Bound
Constant	0.40	0.05		7.38	<0.001	0.27	0.47
Hair cortisol	-0.10	0.02	-0.41	-3.89	<0.001	-0.14	-0.04
Caries experience	-0.01	0.01	-0.09	-0.85	0.397	-0.03	0.01

Calculated using linear regression model with GR $\alpha$  mRNA levels ( $1/\Delta Ct$ ) as the dependent variable.

Constant represents the slope intercept form of the regression equation.

B (unstandardized coefficient) represents the slope of the regression line.

Beta (standardized coefficient) represents the strength of the relationship in the regression model.



**Figure 6.4: Pearson correlation between GR $\alpha$  mRNA levels and hair cortisol levels. Solid line represents the best fit, dashed vertical line represents the cut-off between normal and high cortisol levels (153 ng/g). There was a negative correlation between GR $\alpha$  mRNA levels and hair cortisol levels ( $r = -0.476$ ,  $n = 78$  participants,  $p < 0.001$ ).**

## 6.5 Discussion

The association between stress and dental caries has been studied in different ways and this study aimed to build upon our previous work exploring the association between dental caries experience and hair cortisol as a marker of chronic stress [Abouseta et al., 2024]. Given that individuals with caries experience exhibit a higher level of cortisol than those without [Tikhonova et al., 2018; Nakano et al., 2022], the present study aimed to explore the possible association of stress and dental caries experience with GR receptor mRNA levels in saliva.

Despite numerous studies investigating the expression of GR receptors in various human tissues [Pujols et al., 2002; Melo et al., 2004], the presence of mRNA encoding GR in saliva is relatively unexplored. The present study employed whole saliva pellets as the source for assessing GR mRNA. This choice was grounded in the current literature indicating the presence of human mRNAs in saliva, their stability, and amplifiability,



rendering them suitable as biomarkers for diverse clinical and diagnostic purposes [Park et al., 2006]. Utilization of saliva pellets enabled us to overcome challenges associated with blood sampling and effectively address the research questions at hand [Bonne and Wong, 2012].

RNA can enter the oral cavity through several routes. One source is the secretion of saliva from the three main salivary glands and minor glands. The origin of RNA in salivary secretions can be attributed to either the acinar cells or the circulation [Park et al., 2006]. In this regard, mRNA encoding GR has been identified in human salivary glands [Basarrate et al., 2023]. RNA is also found in gingival crevicular fluid, through which blood cells can enter the oral cavity [Park et al., 2006; Palanisamy and Wong, 2010]. As well, blood cells and circulating RNA can be introduced into the saliva through micro-wounds in the oral cavity. Finally, cells from the oral epithelium are shed into the saliva. In this regard, respiratory epithelial cells have been shown to express GR $\alpha$  [Pujols et al., 2001]. However, the precise source of mRNA encoding GR $\alpha$  in saliva is yet to be determined.

Although some studies have indicated that certain macromolecules in saliva offer protection against ribonucleases [Park et al., 2006; Fábryová and Celec, 2014], it remains crucial to employ proper collection and preservation techniques to maintain RNA integrity [Gandhi et al., 2020]. Salivary RNA is susceptible to degradation by nucleases [Pandit et al., 2013]. As a result, isolating good quality RNA from saliva is difficult and can be costly, primarily due to the cost of commercial kits [Debey-Pascher et al., 2009], which often yield limited amounts of RNA. Our protocol was modified from a previous RNA extraction procedure that used TRIzol to extract RNA from the saliva pellet [Gandhi et al., 2020]. The salivary GAPDH values obtained in the present study are consistent with previous findings [Pandit et al., 2013; Gandhi et al., 2020; Ostheim et al., 2020]. Our results confirm that it is possible to extract RNA from saliva that is amenable to reverse transcription-polymerase chain reaction.

Chronic stress has the potential to induce an allostatic response, disrupting the natural circadian rhythm of cortisol secretion that affects immune responses [McEwen, 1998; Park et al., 2021]. It can potentially increase an individual's susceptibility to oral diseases such as dental caries, periodontal diseases, and bruxism [Boyce et al., 2010; Sabbah et al., 2018;

Gomaa et al., 2020]. Hair cortisol has been previously shown to be a good marker of long term stress [Russell et al., 2012; Gomaa et al., 2020] and to be associated with dental caries [Tikhonova et al., 2018; Nakano et al., 2022; Abouseta et al., 2024]. These previous studies highlighted the need for a deeper understanding of the interaction between the HPA axis and dental caries. In this regard, stress activates the HPA axis and the sympathetic nervous system, which reduces salivary flow rate [Keremi et al., 2017; Bulthuis et al., 2018; Tikhonova et al., 2018; Gomaa et al., 2019] leading to modifications in salivary composition and function.

Glucocorticoids function through binding to the GR, which is widely expressed throughout the body and can initiate a broad range of responses in various cell types and tissues, each with varying degrees of strength and specificity [Ramos-Ramírez and Tliba, 2021]. The variation in cellular responses mediated by the GR is enhanced by the presence of GR isoforms, GR $\alpha$  and GR $\beta$  [Cruz-Topete and Cidlowski, 2014]. Our study found minimal levels of GR $\beta$  mRNA in saliva samples, in line with previous research that indicated limited GR $\beta$  expression in most cells and tissues [Honda et al., 2000; Pujols et al., 2002]. It is suggested that the primary splicing pathway prioritizes the production of GR $\alpha$  mRNA [Oakley et al., 1996]. This could be one of the reasons why the samples from our study did not show GR $\beta$  mRNA.

Numerous studies have reported associations between GR expression and negative feedback inhibition of the HPA axis. These studies indicate that reduced GR $\alpha$  transcription is linked to decreased negative feedback efficacy and increased sensitivity to stress, consequently leading to adverse health outcomes [Weaver et al., 2004; McGowan et al., 2009; Jimeno and Zimmer, 2022]. This decrease is believed to result from chronic exposure to elevated cortisol levels. In this regard, glucocorticoids appear to inhibit GR transcription, likely via the AP-1 and/or AP-2 transcription factors [Bamberger et al., 1996].

Our results are in line with previous findings showing that individuals with high cortisol levels have lower levels of GR $\alpha$  mRNA than those with normal cortisol levels. Our findings also support previous research linking reduced GR $\alpha$  expression to chronic stress [Matsubara et al., 2006; Miller and Chen, 2006; Marin et al., 2007; Panagiotou et al., 2021]. This phenomenon may increase the risk of developing stress-related disorders such as

depression, anxiety, and post-traumatic stress disorder [McGowan et al., 2009; Carvalho et al., 2014]. It may also contribute to metabolic disorders, dysregulated immune responses, chronic inflammation, and increased susceptibility to infections and autoimmune diseases [Kurokawa et al., 2011; Panagiotou et al., 2021].

Our linear regression model showed a significant negative relationship between hair cortisol and GR $\alpha$  mRNA but not dental caries experience. Interestingly, the association between hair cortisol and GR $\alpha$  mRNA levels was significant in the high cortisol group (>153 ng/g), but not in the normal cortisol group. Thus, other factors may play a primary role in regulating GR $\alpha$  expression when cortisol levels are normal. In contrast, chronic stress – resulting in elevated hair cortisol levels – appears to influence glucocorticoid receptor gene expression. On the other hand, caries experience did not exhibit an association with GR $\alpha$  expression. Further research and exploration are needed to understand the underlying mechanisms and implications of these associations in the context of oral health.

It is essential to acknowledge the limitations of this study, including its cross-sectional nature, which precludes establishing causality. Additionally, potential confounding variables, such as lifestyle factors, should be considered in future research to refine the understanding of the relationship between GR $\alpha$  expression, stress, and caries. One of the research limitations is the absence of standardized protocols for collecting and extracting RNA from saliva, making it difficult to compare and validate results from independent laboratories. This lack of standardization can also introduce potential sources of bias and variability in the data. Therefore, it is difficult to compare our results with those from other sources of mRNA such as blood or tissue samples.

## 6.6 Conclusions

To the best of our knowledge, this is the first report of the presence of GR $\alpha$  mRNA in saliva. There is a significant negative association between hair cortisol levels and GR $\alpha$  mRNA levels. In contrast, there is no significant difference in salivary GR $\alpha$  mRNA between individuals with caries experience and those without. In addition, there is reduced expression of GR $\alpha$  in individuals with high hair cortisol levels and caries experience, when

compared to individuals with normal hair cortisol levels with and without caries experience. The link between GR $\alpha$  mRNA levels and hair cortisol levels suggests that salivary GR $\alpha$  levels could be explored as potential biomarker for chronic stress. A thorough understanding of the intricate relationships among stress, GR $\alpha$ , and oral health may facilitate the development of targeted interventions to improve oral and overall health outcomes.

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## Chapter 7

### 7 Conclusions and General Discussion

#### 7.1 Conclusions

The present work focused on the relationships among social factors, for example, socioeconomic status, demographic and psychological factors, and oral health outcomes in adolescents and young adults at individual and neighborhood levels. The primary objective was to investigate social and biological factors that connect socioeconomic backgrounds to oral health outcomes and dental caries experience. The study employed primary data (individual level) and secondary data (neighborhood level) to address the following research questions: Are there are socioeconomic differences in dental treatment utilization in adolescents and young adults? Is there a relationship between cortisol levels (in hair and saliva) and caries experience, and is it associated with individuals facing social adversity? Could mRNA encoding glucocorticoid receptors be present in saliva samples, and are their levels related to chronic stress and dental caries experience in adolescents and young adults? The thesis research addressed these questions through three projects, which led to the following conclusions:

**Project 1:** Neighborhood-Level Inequalities in Dental Care of Adolescents and Young Adults in Southwestern Ontario (Chapter 4)

Objective: Examine whether the association of neighborhood-level socioeconomic status with the cost of dental care and dental care outcomes differs between adolescents and young adults.

Conclusions:

- Young adults have lower preventive services and higher dental treatment services scores when compared to adolescents.
- A negative association exists between dental treatment services and neighborhood-level median household income in adolescents and young adults.

- Individuals from neighborhoods with lower household incomes show a higher cost of dental care, yet worse treatment outcomes.

**Project 2: Relationships among Cortisol, Perceived Stress, and Dental Caries Experience in Adolescents and Young Adults (Chapter 5)**

Objective: Assess the association among perceived stress, cortisol levels (in hair and saliva), and the overall caries experience of adolescents and young adults aged between 15 and 25 years.

Conclusion: Hair cortisol levels and perceived stress are to be significantly associated with the experience of dental caries, while salivary cortisol concentrations do not show any such correlation.

**Project 3: Profiling mRNA encoding glucocorticoid receptor  $\alpha$  in saliva: Relationship to hair cortisol levels in individuals aged 15-25 years (Chapter 6)**

Objective: Compare GR $\alpha$  transcript levels between those individuals with and without dental caries experience, as well as between individuals with and without elevated hair cortisol levels

Conclusions:

- mRNA encoding GR $\alpha$  is present in saliva.
- There is no significant difference in levels of salivary GR $\alpha$  mRNA between individuals with caries experience and those without.
- There is a significant negative association between hair cortisol levels (a marker of chronic stress) and GR $\alpha$  mRNA levels in saliva.
- Thus, salivary GR $\alpha$  mRNA levels could be investigated as a potential biomarker for chronic stress.

## 7.2 Neighborhood Level Demographics and Dental Care Costs

Utilizing healthcare services is crucial to ensuring the maintenance of ideal health. Canada's universal healthcare system covers most medical care for its citizens. Conversely, dental care has been primarily privately financed privately [Martin et al., 2018]. Private financing covers 94% of dental care expenses, with the remaining 6% being funded publicly. Among the privately financed portion, 60% is covered by employer-based insurance, while 40% is paid directly by individuals out-of-pocket [Quiñonez et al., 2021]. Recently, the Canadian government has revealed specifics about the long-awaited Canadian Dental Care Plan (CDCP), which is expected to enhance access to dental care for approximately 9 million uninsured Canadians with annual household incomes below \$90,000.

In Ontario, the Healthy Smiles Program is a free dental program for eligible children and youth 17 and under [Healthy Smiles Ontario, 2016]. Young adults 18 and over from low-income households must pay from their out-of-pocket. Our study analyzed a diverse sample of 2,915 patients from the London, Ontario metropolitan area, with an average age of 19.7 years. We observed significant differences in dental care costs, with young adults incurring notably higher dental care expenses when compared to adolescents (Chapter 4). Our findings were consistent with recent research conducted in Ontario, which revealed that young adults aged 20-39 face significant cost barriers to dental care [Abdelrehim et al., 2023]. This suggests that young adults may experience challenges in accessing dental care compared to other age groups, potentially leading to avoidance of dental visits.

Furthermore, our study in Chapter 4 showed that individuals from neighborhoods with higher median household incomes had lower average dental care costs per person than those from neighborhoods with lower median household incomes. This pattern was observed in both the 15–17 and 18–24 age groups, aligning with previous research findings [Thompson et al., 2014; Zivkovic et al., 2020]. This indicates that individuals from lower-income families may experience higher dental care expenses.

These findings underscore the significance of cost as a barrier to dental care in Canada and highlight the substantial financial impact on many households seeking dental services [Nyamuryekung'e et al., 2019]. Our research revealed that preventive dental services were linked to a significant reduction in costs for young adults. This aligns with previous studies showing that regular preventive dental care can significantly decrease overall dental treatment expenses [Fraihat et al., 2019; Nyamuryekung'e et al., 2019].

### 7.3 Neighborhood Level Social Inequalities in Dental Care Outcomes

Socioeconomic disparities in oral health outcomes at the neighborhood level pose a public health challenge with negative impacts on individuals and society [Peres and Heilmann, 2015; Peres et al., 2019]. To gain a more thorough comprehension of the disparities in dental care outcomes, the study in Chapter 4 employed four neighborhood-level variables as predictors. These variables included median household income, educational attainment, primary language spoken at home, and recent immigration. In neighborhoods with higher household incomes, there was a clear trend where residents tended to receive more preventive care and required less treatment. This observation highlights the significant role of socioeconomic disparities in shaping oral health outcomes, especially during the transition into young adulthood.

Regarding dental treatment, we found a significant inverse association between the median household income at the neighborhood level and the utilization of dental treatment services in both age groups. This suggests that families with higher median incomes are at a lower risk of requiring dental treatment. Overall, our results support the well-established idea that individuals from families with lower socioeconomic status are more likely to experience adverse oral health outcomes [Schwendicke et al., 2015; Trohel et al., 2016; Mehra et al., 2019]. These findings indicate that lower socioeconomic status is associated with poorer oral health outcomes, and that the association of median household income with oral health outcomes is particularly pronounced in the 18-24 age group.

## 7.4 Geographic Patterns of Dental Care Outcomes

A software package ArcGIS Pro (Esri Canada, Vancouver, Canada) was used to create maps to identify target populations within Southwestern Ontario that display better or worse dental treatment outcomes when matched for sociodemographic variables. Geovisualization of dental care outcomes revealed significant variations. Although there was an overall increase in the utilization of routine dental examinations among young adults compared to adolescents, this was not similar across all regions. This non-uniform distribution suggests the existence of geographic inequalities in access to routine dental care. Additionally, certain areas displayed a decrease in preventive service scores and a simultaneous increase in treatment service scores for young adults when compared to adolescents. These observed geographic patterns warrant further investigation to determine the underlying factors contributing to disparities in access to and utilization of dental care.

## 7.5 Caries Experience and the Socioeconomic Status of Individuals

The dissertation adopted a stratification-based method to investigate the relationship between family socioeconomic status and caries experience. These outcomes were assessed using various indicators, including family income, parental education, insurance, dental visits, and immigration status. Individuals with lower household income and those lacking dental insurance coverage were more likely to have caries experience. Our findings are in line with the results of the Canadian Health Measures Survey (2007 - 2009), which showed that lower-income individuals, who lack dental insurance, avoid dental visits due to the cost [Cooney, 2010; Levy et al., 2023].

Additionally, parental education and dental visits per year were found to be associated with caries experience. Our results agree with the literature [Pattussi et al., 2001; Reisine and Psoter, 2001; Boyce et al., 2010; Peres and Heilmann, 2015; Steele et al., 2015; Zangiabadi et al., 2017; Singh et al., 2019], which shows that individuals from lower socioeconomic backgrounds tend to experience poorer oral health outcomes.

While the various indicators of socioeconomic status may exhibit comparable associations with caries experience, it is imperative to recognize that each indicator functions through

distinct mechanisms that impact the development of dental caries. This recognition is essential for developing targeted interventions and policies to address oral health disparities effectively. Diverse indicators of socioeconomic status could encompass separate social processes and pathways that contribute to oral health inequalities. Hence, incorporating multiple indicators enables a more refined and precise comprehension of the intricate interplay between socioeconomic factors and oral health [Galobardes et al., 2006; Steele et al., 2015]. Our results were consistent with previous work and aligned with the general susceptibility hypothesis of disease etiology, which holds that people who experience adverse social circumstances may be more susceptible to illness than those who live in wealthy environments.

Comparing the findings of this dissertation with existing studies is challenging due to the substantial heterogeneity in the outcomes assessed, age groups studied, and explanatory variables considered. However, it is crucial to consider the impact that social factors during childhood can have on oral health later in life [Poulton et al., 2002; Peres et al., 2007; Heilmann et al., 2015].

## 7.6 The Role of Psychological Factors in Caries Experience Related Social Inequalities

The psychosocial perspective suggests that an individual's psychological well-being is influenced by their social status, and this influence follows a social gradient. Consequently, psychosocial factors were found to be independently linked to oral health outcomes [Gomaa et al., 2019]. Specifically, our study revealed that disparities in dental caries experience among socioeconomic groups were associated with elevated perceived stress, unmanageable stress, and financial barriers to accessing dental care.

Earlier studies established positive associations between socioeconomic status, heightened financial stress, increased levels of stress hormones, and cariogenic bacterial counts, contributing to the development of dental caries [Silva et al., 2018; Tikhonova et al., 2018; Nakano et al., 2022]. In the context of periodontal disease, stress-induced cortisol release was theoretically suggested to reduce inflammation in the gums, potentially mitigating the severity of the condition [Obulareddy et al., 2018; Castro et al., 2020; Gomaa et al., 2020].

However, it should be noted that chronic stress can adversely affect overall health, including the immune system [Sabbah et al., 2018].

Considering the given information, our proposed research model sought to enhance understanding of the biological pathways involved in caries experience. To achieve this, we excluded individuals diagnosed with periodontal disease from our study sample. Our work is the first to show a relationship between hair cortisol and caries experience while controlling gingival bleeding. On the other hand, salivary cortisol was not correlated with caries experience, which is consistent with some earlier research findings [Yfanti et al., 2014; Tikhonova et al., 2018].

The present research found that high cortisol levels in hair were associated with caries experience. These findings conform to the general susceptibility view of disease causation – prolonged exposure to chronic stress is linked to increased likelihood of developing chronic illnesses. This link may contribute to the connection between exposure to adverse socioeconomic conditions and negative health outcomes. When the body is subjected to chronic stress without respite, it can lead to the suppression of the immune system, eventually resulting in the manifestation of various illnesses [Salleh, 2008; Sabbah et al., 2018; Gomaa et al., 2020; Crielaard et al., 2021].

Previous research indicated that perceived stress is related to dental caries experience [Beck, 1987; Hugo et al., 2007; Jain et al., 2014; Dahl et al., 2018; Kadhum and Qasim, 2020; Yang et al., 2022]. Our study supported this finding by revealing that participants with higher perceived stress scale (PSS) scores were more likely to have experienced dental caries than those with low PSS stress scores.

## **7.7 Activation of the Stress Pathway is Linked to Caries Experience**

Our study in Chapter 5 found that chronic physiological stress, as indicated by elevated hair cortisol levels, is strongly associated with increased risk of having experienced dental caries. Similarly, psychological stress, as measured by the PSS score, is correlated with a greater likelihood of dental caries. Notably, these associations remained robust even after



accounting for potential confounding factors in the multivariable analysis. The findings highlight the importance of addressing physiological and psychological stress in comprehensive oral health interventions, and the need for further research to explore underlying mechanisms and interventions for stress-related dental caries.

## 7.8 Salivary Levels of mRNA Encoding the Glucocorticoid Receptor and their Association with Cortisol Levels and Caries Experience

In Chapter 5, we showed an association between stress and caries experience in adolescents and young adults. Although dental caries is primarily a disease related to diet, several factors can influence the progression of the disease, including host factors [Selwitz et al., 2007; Opal et al., 2015]. Certain psychosocial and biological factors have been found to modify these host factors [Finlayson et al., 2007; Tikhonova et al., 2018; Kadhum and Qasim, 2020]. Still, they do not fully explain the oral health disparities observed in different social groups. Some researchers have used salivary and hair cortisol to explore this issue [Pani et al., 2013; Lee et al., 2015; Tikhonova et al., 2018; Anand et al., 2020]. The limitations of these approaches have been documented [Ayhan et al., 1996; Rai et al., 2010; Tikhonova et al., 2018]. Recent evidence indicates that expression of the glucocorticoid receptor can provide an acceptable measure of activation of the HPA axis [Gjerstad et al., 2018; Vassiliou et al., 2019; Jimeno and Zimmer, 2022].

To the best of our knowledge, Chapter 6 is the first study to investigate mRNA encoding GR in saliva samples. Our results indicated that GR $\alpha$  mRNA levels are significantly different between individuals with lower and higher hair cortisol levels. On the other hand, there is no significant difference in GR $\alpha$  mRNA levels between individuals with no caries experience and those who had experienced dental caries.

Individuals with adequate levels of GR expression can efficiently regulate glucocorticoid levels, facilitating adaptation to stressors and helping to cope with environmental challenges [Jimeno and Zimmer, 2022]. Negative feedback is primarily coordinated by glucocorticoids binding to GR in the hippocampus, hypothalamus, and pituitary gland, reducing the secretion of glucocorticoids. Conversely, individuals with suppressed levels

of GR expression may have a reduced capacity to modulate their responses successfully in response to environmental stressors [Jimeno and Zimmer, 2022].

Our linear regression analysis showed a significant inverse correlation between hair cortisol levels and GR $\alpha$  mRNA expression, whereas no significant association was found with dental caries experience. The lack of significance suggests that other factors or pathways play a role in the relationship between stress and dental health. This is consistent with the multifactorial nature of dental caries development [Pattussi et al., 2001; Manton, 2018; Pierce et al., 2019] and the intricate nature of stress-mediated diseases. Reduced GR $\alpha$  expression in individuals with high cortisol levels may be due to the association between GR $\alpha$  and cortisol. However, more research is needed to fully understand the mechanisms and clinical significance of these findings.

## 7.9 Strengths and Limitations

A strength of the thesis research is that it was guided by the integration of community and individual levels of data and analysis related to oral health outcomes. Moreover, the dissertation considered and integrated biological, psychological, and social dimensions of health and illness, aligning with the fundamental principles of the biopsychosocial model. This approach aids in pinpointing potential pathways that contribute to inequalities in oral health. Undoubtedly, multiple factors and intricate layers of complexity associated with oral health disparities necessitate further explanation.

In Chapter 4, the dissertation benefited from measuring the impacts of neighborhood-level social and economic factors on a number of oral health outcomes (routine oral evaluation, utilization of preventive services, and dental treatment services). As well, I used the Dental Quality Alliance protocol for collecting electronic dental billing data, which reflects the quality of dental care received. Using billing data through electronic health records has been shown to indicate both caries risk and access to dental care accurately [Ojha and Aravamudhan, 2016; Podskalniy et al., 2021]. This comprehensive approach is valuable for understanding the relationships among stress, caries experience, and dental care utilization within a broader context of oral health.

Combining questionnaires and biological samples like hair and saliva is an effective way to collect comprehensive data (Chapters 5 and 6). The approach captures subjective self-reports and objective biological markers, providing an integrative analysis and a deeper understanding of various factors influencing oral disease outcomes. Combining quantitative and qualitative data promotes a more holistic understanding of the complex interplay between psychosocial factors and oral health outcomes. The continuity in using the same samples, particularly in Chapters 5 and 6, ensured the consistency and reliability of the research findings.

By revealing the association between hair cortisol and caries experience, we contributed to understanding the complex interplay between psychosocial factors and biological mechanisms in oral health. This finding may provide the basis for future studies exploring how stress is related to dental health outcomes.

An additional strength of this work is that the choice of the age group, adolescents, and young adults (15 to 25 years of age). This age group has historically not received adequate attention in oral health research, or preventive programs compared to other demographic segments.

Our study in Chapter 6 expanded research on oral biomarkers associated with stress by extracting RNA from whole saliva and utilizing qPCR to evaluate levels of GR mRNA. Our study strengthens the potential for saliva as a non-invasive biomarker and reliable source for RNA extraction. The presence of GR $\alpha$  mRNA in saliva samples opens new avenues for research in the fields of stress biology and biomarker development.

Along the course of this work, there were several limitations to our study, some of which have already been noted in Chapters 4, 5 and 6.

A common limitation of this thesis is the use of a convenience sample. In Chapter 4, we focused on individuals who visited subsidized clinics of a dental school, which might not be representative of the entire population. Moreover, the inclusion of only 17 FSA codes made it impossible to apply more rigorous neighborhood-level regression modeling, such as the ordinary least squares (OLS) model. To address this limitation, future work should

aim to increase the sample size by including subjects from various sources. These could include private clinics and community health centers, which would help to incorporate individuals from different socioeconomic backgrounds and geographic locations to capture a more diverse population.

Biological studies are often subject to certain limitations that can affect the accuracy and reliability of findings. One of the major limitations is the presence of physiological variations that can occur between different subjects or even between samples collected from the same individual in different situations. These variations can make it difficult to draw conclusions that are applicable to a larger population. To minimize these variations in our studies, we implemented a standardized protocol for collecting saliva samples with special attention to the time of collection.

Another drawback is the limited number of participants, which can lead to underpowered studies that are not able to detect significant differences or associations. Our sample collection was challenged by COVID restrictions. These included enhanced safety policies, travel restrictions, social distancing measures, as well as reduced clinic space which limited our access to study participants for data collection.

The cross-sectional design can provide valuable snapshots of associations but does not establish causality. Future longitudinal or interventional designs might be needed to determine causal relationships. Furthermore, the modest sample size in Chapters 5 and 6 can impact the generalizability of our findings. A larger and more diverse sample would improve the external validity of the results.

The study sample in Chapters 5 and 6 was recruited from Schulich dental clinics and again does not accurately represent the general population. Therefore, caution should be exercised when interpreting the findings as they may not be applicable to the wider population.

The reliance on self-reported data, such as stress levels, socioeconomic status, and dental care utilization, can be subject to recall bias and may not always accurately reflect the true behaviors and experiences of participants. Moreover, our study sample was unequally

distributed with respect to socioeconomic status with more participants having low incomes. This distribution is anticipated due to the subsidized nature of the dental school clinics involved in the study.

## 7.10 Future Directions

Future research could improve upon these limitations in several ways. Studies could be conducted in a broader range of geographic areas, encompassing more FSA codes to increase the representativeness of the findings. Additionally, including data from private dental clinics and other healthcare settings would help to capture a more diverse patient population.

Implementation of longitudinal research designs could shed light on causal relationships among stress, socioeconomic factors, dental health, and healthcare utilization. Moreover, it might be helpful to incorporate additional measures of stress, such as parental stress. This stress arises from parental socioeconomic status, psychosocial factors, and environmental factors. Parental stress may in turn affect the general and oral health of their children, including adolescents.

Further research is required to establish the validity of our findings regarding the relationship between GR mRNA levels and hair cortisol levels, as well as caries experience. It is essential to consider other biological factors that could provide a more comprehensive understanding of social-biological interactions in oral diseases. These could include investigating epigenetic modifications in genes associated with GR and caries. Additionally, examining the composition and diversity of the oral microbiome in relation to cortisol levels and GR mRNA would be of interest.

## 7.11 Concluding Remarks

Limited information is available regarding the oral health of Canadian adolescents and young adults, with most research focusing on either children or adults. However, maintaining good oral health during these crucial ages is imperative, given the heightened risk for certain oral diseases in this age period.

In our studies, young adults exhibited poorer preventive and treatment outcomes than adolescents. Individuals from lower-income neighborhoods also faced significantly higher dental care costs and worse treatment outcomes. Interestingly, no relationships were found between saliva cortisol concentration and caries experience, but hair cortisol levels and perceived stress scores showed significant associations with caries experience. Notably, individuals with high cortisol levels demonstrated significantly lower GR $\alpha$  mRNA expression than those with normal hair cortisol levels.

The biomarkers explored in this study constitute physiological measures that can contribute to future research on chronic stress and its impact on oral health. Although the field is progressing, additional research on refining measurement methodologies is necessary. Achieving a more profound and nuanced understanding of these complex physiological pathways holds the potential to inspire future researchers. This enhanced understanding, in turn, can pave the way for the formulation of interventions and policies aimed at improving health outcomes and reducing disparities among adolescents and young adults grappling with chronic stress.

## 7.12 References

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## Appendices

### Appendix 1: Advertisement for Participant Recruitment

#### Research Study



#### The link between Stress and Oral Health in Young Adults



#### **PARTICIPANTS NEEDED**

for a study on the effect of stress on oral health

- Age -15-25 years
- Participants will receive a dental examination and individualized oral hygiene instructions
- **Gift card** to compensate for your time

To find out if you are eligible and to learn more about the study, please call

[REDACTED]  
[REDACTED]

## **Appendix 2: Children's Consent Form**

### **Part I: Information letter**

Study title: Long-term stress, geographic indicators, and sociodemographic variables as predictors of dental treatment outcomes in adolescents and young adults.

Principal Investigator Contact

Dr. Sharat Chandra Pani

Assistant Professor of Paediatric Dentistry

Schulich School of Medicine and Dentistry

Office [REDACTED]

Western University, London, Ontario, Canada [REDACTED]

Phone: [REDACTED]

Additional Research Staff

Naima Abouseta, PhD student

Source of Funding: Research Grant from the School of Dentistry, Schulich School of Medicine and Dentistry

Introduction:

You are being invited to take part in a research study at Western University, funded through an internal grant from the Schulich School of Medicine & Dentistry. Please note that participation in this study is completely voluntary. This consent form provides you with information to help you make an informed choice. Please read this document carefully and take your time in making your decision. You can always ask the study doctor to explain anything that you do not understand and make sure that all your questions have been answered before signing this consent form. Before you make your decision, you may find it helpful to discuss it with your friends and family.

If you have any questions that come up later, you can contact the study doctor via the email address or telephone number provided under the contact section of this form.

Background and Purpose:

Oral disease is one of the most prevalent chronic diseases in Canada, with its burden concentrating largely on Canadian families of lower income and education, who typically lack dental insurance and avoid dental visits because of costs. This is unfair and due to the social and living conditions. Previous studies have shown oral diseases such as gum disease and dental decay, to be linked to stress, which affects the body's different systems including hormonal balance and the ability of the body to stand off disease.

Our study aims to understand how social and general life conditions affect oral health and lead to oral disease.

This study consists of two parts; the first part of the study is a questionnaire aimed at collecting socioeconomic status, self-rated general and oral health, access to medical and dental health services, and perceived stress scales.

In the second part, we will examine the relationship between social factors such as income, education, and stressful life events with oral health status and investigate the stress hormone levels in saliva and hair. In addition, we will also study if the latter relates to the stress gene in the saliva sample.

#### Participant selection

We are inviting adolescents and adult males and females (15 to 24 years old) to participate in this study. Only residents of Southwest Ontario from whom valid informed consent will be obtained are included in this study. Further inclusion criteria will include willingness to provide a hair sample, sufficient hair growth at the posterior vertex position of the head, and a hair sample weight of at least 5 mg.

Exclusion criteria: Gingival inflammation/bleeding during the oral examination; current chronic, autoimmune, or inflammatory disease; prolonged corticosteroid, antibiotic, or prescription medication use; current depressive or anxiety disorder; or presence of orthodontic appliances.

#### Study Procedures:

If you agree to participate in the study:

- 1- You will be asked to complete a medical and dental history an online questionnaire related to your health, your social and living conditions, and your daily life stressors which is designed to take no longer than 7-10 minutes.
- 2- You will then be asked to collect your saliva secretion and spit about 5-10 ml of oral fluid in a tube. Before the collection, you will be instructed not to eat or drink for a minimum of 1 hour.
- 3- You will be asked to provide approximately 100 hairs from the back of the head, in a painless, safe, and non-invasive procedure, using sterile scissors. No hair will be pulled out.
- 4- You will then have a complete dental examination similar to a regular dental examination at your dentist.
- 5- The findings of your dental examination will be discussed with you and if any oral problems are identified, you will be advised of options regarding follow-up and treatment. In addition, you will receive individualized instructions in oral hygiene (i.e. proper technique of brushing and flossing).
- 6- The entire procedure should take between 1.5-2 hours.

#### Risks:

There is no foreseeable harm or injury as a result of participating in this study.

#### Benefits:

There are no direct benefits to your participation in this study.

**Confidentiality:**

If you agree to take part in this study, the information that will be collected for this project will be kept under strict confidentiality. Information about you will be anonymized and will be locked and secured onto a protected desktop on a secure server by the study doctor for 7 years. This information may be used in further studies of similar research purposes to build up on the findings from this study. All collected data will be identified with a unique study ID only. Your information will not be shared with third parties. The data that will be shared on any database or publications will not contain any information that can identify you. We would like to state that as personal identifiers are being collected as per this study, there is the risk of breach of privacy.

Also, identifiable study data may be accessed by the Western University Health Sciences Research Ethics Board to monitor the conduct of this project.

**Voluntary Participation:**

Taking part in this study is completely voluntary. You may choose not to take part, or if you choose to participate, you may leave the study at any time without giving a reason. You are deciding not to take part, or deciding to leave the study later, will not result in any penalty or any loss of benefits to which you are entitled. If you already are a dental clinic patient, all the services you receive at this clinic will continue and nothing will change. If you decide to withdraw from the study, all samples and related study data will also be destroyed.

**Duration:**

You are required to come for a single appointment, two hours in length, to which personal information, sample collection, and oral examination will take place.

**Costs:**

Taking part in this study should not result in added costs to you. Examples of possible additional costs may include costs associated with internet and computer access.

**Compensation:**

You will not be paid for taking part in this study. However, all participants will be given the option to get a free dental examination and cleaning. In addition, participants will be given a 20\$ gift card for their participation in the study.

**Sharing the Results:**

You are always welcome to contact the study doctor within 12-18 months from the date of your appointment, via telephone to email, to learn more about the study results.

**Conflict of Interest:**

There is no conflict of interest to disclose.

**Questions About the Study?**

If you have any questions or concerns now or later or would like to speak to the study team for any reason, please call the study doctor, Dr. Sharat Pani, Schulich School of Medicine and Dentistry, Western University, Phone: [REDACTED]

**Part II: Written Consent**

Project Title: Long-term stress, geographic indicators, and sociodemographic variables as predictors of dental treatment outcomes in adolescents and young adults.

Document Title: Letter of Consent

Principal Investigator + Contact

Dr. Sharat Pani

Schulich School of Medicine and Dentistry, Western University

Email: [REDACTED]

Phone: [REDACTED]

Researcher: Naima Abouseta

Schulich Dentistry

Email: [REDACTED]

I have read the foregoing information. I have had the opportunity to ask questions about it, and the questions that I have asked to have been answered to my satisfaction. I understand that my anonymized information will be retained and may be used for future studies. I consent voluntarily to take part as a participant in this study.

Consent

Child's Name: \_\_\_\_\_

Parent / Legal Guardian (Print): \_\_\_\_\_

Parent / Legal Guardian (Sign): \_\_\_\_\_

Parent / Legal Guardian (Date): \_\_\_\_\_

This letter is yours to keep for future reference.



### **Appendix 3: Adult Consent Form.**

#### Part I: Information letter

Study title: Long-term stress, geographic indicators, and sociodemographic variables as predictors of dental treatment outcomes in adolescents and young adults.

#### Principal Investigator Contact

Dr. Sharat Chandra Pani

Assistant Professor of Paediatric Dentistry

Schulich School of Medicine and Dentistry

Office [REDACTED]

Western University, London, Ontario, Canada [REDACTED]

Phone: [REDACTED]

#### Additional Research Staff

Naima Abouseta, PhD student

Source of Funding: Research Grant from the School of Dentistry, Schulich School of Medicine and Dentistry

#### Introduction:

You are being invited to take part in a research study at Western University, funded through an internal grant of the Schulich School of Medicine & Dentistry. Please note that participation in this study is completely voluntary. This consent form provides you with information to help you make an informed choice. Please read this document carefully and take your time in making your decision. You can always ask the study doctor to explain anything that you do not understand and make sure that all your questions have been answered before signing this consent form. Before you make your decision, you may find it helpful to discuss it with your friends and family.

If you have any questions that come up later, you can contact the study doctor via the email address or telephone number provided under the contact section of this form.

#### Background and Purpose

Oral disease is one of the most prevalent chronic diseases in Canada, with its burden concentrating largely in Canadian families of lower income and education, who typically lack dental insurance and avoid dental visits because of costs. This is unfair and due to the social and living conditions. Previous studies have shown oral disease such as gum disease

and dental decay, to be linked to stress, which affects the body's different systems including hormonal balance and the ability of the body to stand off disease.

Our study aims to understand how social and general life conditions affect oral health and leading to oral disease.

This study consists of two parts; the first part of the study is a questionnaire aimed at collecting socioeconomic status, self-rated general and oral health, access to medical and dental health services and perceived and financial stress scales.

The second part we will examine the relationship between social factors such as income, education and stressful life events with the oral health status and investigate the stress hormone levels in saliva and hair. In addition, we will also study if the latter relates to stress gene in the saliva sample.

#### Participant selection:

We are inviting adolescents and adult males and females (15 to 24 years old) to participate in this study. Only residents of Southwest Ontario from whom valid informed consent will be obtained are included in this study. Further inclusion criteria will include willingness to provide a hair sample, sufficient hair growth at the posterior vertex position of the head, and a hair sample weight of at least 5 mg.

Exclusion criteria: Gingival inflammation/bleeding during the oral examination; current chronic, autoimmune, or inflammatory disease; prolonged corticosteroid, antibiotic, or prescription medication use; current depressive or anxiety disorder; or presence of orthodontic appliances.

#### Study Procedures:

If you agree to participate in the study:

- 1- You will be asked to complete a medical and dental history an online questionnaire related to your health, your social and living conditions and your daily life stressors which is designed to take no longer than 7-10 minutes.
- 2- You will then be asked to collect your saliva, and spit about 5-10 ml of oral fluid in a tube, before the collection, you will be instructed not to eat or drink for a minimum of 1 hour.
- 3- You will be asked to provide approximately a hair sample (approximately 100 hairs  $\geq$ 20 mg of hair) of at least 3 cm in length will be collected at the back of the head, in a painless, safe and non-invasive procedure, using sterile scissors. No hair will be pulled out.
- 4- You will then have a complete dental examination similar to a regular dental examination at your dentist.

5- The findings of your dental examination will be discussed with you and if any oral problems are identified, you will be advised of options regarding follow-up and treatment. In addition, you will receive individualized instructions in oral hygiene (i.e. proper technique of brushing and flossing).

6- The entire procedure should take between 1.5-2 hours.

**Risks:**

There is no foreseeable harm or injury as a result of participating in this study.

**Benefits:**

There are no direct benefits to your participation in this study.

participants may experience no benefit from participation.

**Confidentiality:**

If you agree to take part in this study, the information that will be collected for this project will be kept under strict confidentiality. Information about you will be anonymized and will be locked and secured onto a protected desktop on a secure server by the study doctor for 7 years. This information may be used in further studies of similar research purposes to build up on the findings from this study. All collected data will be identified with a unique study ID only. Your information will not be shared with third parties. The data that will be shared on any database or publications will not contain any information that can identify you. We would like to state that as personal identifiers are being collected as per this study, there is the risk of breach of privacy. Also, as that identifiable study data may be accessed by Western University Health Sciences Research Ethics Board to monitor the conduct of this project.

**Voluntary Participation:**

Taking part in this study is completely voluntary. You may choose not to take part, or if you choose to participate, you may leave the study at any time without giving a reason. You are deciding not to take part, or deciding to leave the study later, will not result in any penalty or any loss of benefits to which you are entitled. if you already are a dental clinic patient, all the services you receive at this clinic will continue and nothing will change. If you decide to withdraw from the study, all samples and related study data will also be destroyed.

**Duration:**

You are required to come for a single appointment, two hours in length, in which personal information, sample collection and oral examination will take place.

**Costs:**

Taking part in this study should not result in added costs to you. Examples of possible additional costs may include costs associated with internet and computer access.

**Compensation:**

You will not be paid for taking part in this study. However, all participants will be given the option to get a free dental examination and cleaning. In addition, participants will be given a 20\$ gift card for their participation in the study.

**Sharing the Results:**

You can always welcome to contact the study doctor in 12-18 months from the date of your appointment, via telephone to email, to learn more about the study results.

**Conflict of Interest:**

There is no conflict of interest to disclose.

**Questions About the Study?**

If you have any questions or concerns now or later or would like to speak to the study team for any reason, please call the study doctor, Dr. Sharat Pani, Schulich School of Medicine and Dentistry, Western University, Phone [REDACTED]

**Part II: Written Consent**

Project Title: Long-term stress, geographic indicators, and sociodemographic variables as predictors of dental treatment outcomes in adolescents and young adults.

Document Title: Letter of Consent

Principal Investigator + Contact

Dr. Sharat Pani

Schulich School of Medicine and Dentistry, Western University

Email: [REDACTED]

Phone: [REDACTED]

Researcher: Naima Abouseta

Schulich Dentistry

Email: [REDACTED]

I have read the foregoing information. I have had the opportunity to ask questions about it, and the questions that I have asked have been answered to my satisfaction. I understand that my anonymized information will be retained and may be used for future studies. I consent voluntarily to take part as a participant in this study.

Print Name of Participant

Signature of Participant

Date: (DD/MM/YYYY)

\_\_\_\_\_

\_\_\_\_\_

This letter is yours to keep for future reference.

Statement by the researcher/study doctor

I have accurately read out the information sheet to the potential participant, and made sure that the participant understands the procedures, potential risks and benefits. I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

Print Name of Researcher

\_\_\_\_\_

Signature of Researcher

Date: (DD/MM/YYYY)

\_\_\_\_\_

**Appendix 4: Telephone Script (version for participants younger than 18 years)**

“Hello, may I please speak with [insert the name of the parent of potential participant here].”

“Hi, [name of the parent of potential participant here] this is Naima, PhD student and calling from the office of Dr Sharat Pani.

I am calling today because you are listed in Salud database and previously indicated your willingness to be contacted about research opportunities that may involve speaking with [insert name of the potential participant here].

We are conducting a study on of Long-term stress and sociodemographic factors of dental treatment outcomes in adolescents and young adults. Oral examination will be screened of any oral disease. We are looking to collect saliva and hair samples, which are simple, painless and non-invasive procedures. We will be offering you a free dental exam and card gift to compensate for your time.

Would you and/or [insert name of potential participant here] be interested in hearing more about this study?”

If no, thank them for their time and say good-bye\*

\*If yes, continue to explain study details to them based on the Letter of Information\*

\*If after providing study details the participant is still interested, information letter will be sent via email or post for the participant to review prior to being asked to consent or being invited to schedule the first study visit where consent will be obtained.

By the end of the call will ask, “Do you have any questions?”

[Answer any questions they may have]

**Appendix 5: Telephone Script (version for participants 18 years of age and older)**

“Hello, may I please speak with [insert the name of the potential participant here].”

“Hi, [name of the potential participant] this is Naima, PhD student and calling from the office of Dr Sharat Pani.

I am calling today because you are listed in the Salud database and previously indicated your willingness to be contacted about research opportunities.

We are conducting a study on of Long-term stress and sociodemographic factors of dental treatment outcomes in adolescents and young adults. Oral examination will be screened of any oral disease. We are looking to collect saliva and hair samples, which are simple, painless and non-invasive procedures. We will be offering you a free dental exam and card gift to compensate for your time.

Would you be interested in hearing more about this study?”

If no, thank them for their time and say good-bye\*




\*If yes, continue to explain study details to them based on the Letter of Information\*

\*If after providing study details the participant is still interested, information letter will be sent via email or post for the participant to review prior to being asked to consent or being invited to schedule the first study visit where consent will be obtained.

By the end of the call will ask, “Do you have any questions?”

[Answer any questions they may have]

### Appendix 6: Stress and Socioeconomic Position Questionnaire

<p>Now, I will ask you some questions about yourself and your health, it shouldn't take longer than 15 minutes, ok?</p>
<p>What is your current living status?</p> <ol style="list-style-type: none"> <li>1. Single - live alone.</li> <li>2. Married or live with partner.</li> <li>3. Single - live with parent.</li> </ol>
<p>In the past year have your parents supported you in any way financially (either through money for rent, tuition, books or shelter) or have you spent more than a month living in your parents' home(s)?</p> <ol style="list-style-type: none"> <li>1. Yes</li> <li>2. No</li> </ol>
<p>If answer No.</p> <p>We'd like to estimate your total income, before taxes, for the past year. Was it:</p> 
<ol style="list-style-type: none"> <li>1. If the answer Yes.</li> <li>2. What was the total combined income for your household last year (From all sources before taxes)?</li> </ol> 
<p>If you live with partner/ married</p> <p>What was the total combined income for you and your partner last year (From all sources before taxes)?</p> 
<p>How many children under the age of 16 are in your family?</p>
<p>How many people live in your household?</p>
<p>What is the highest level of schooling you have completed?</p> <ol style="list-style-type: none"> <li>1. Primary (0-6 years)</li> <li>2. High school without graduation</li> </ol>



3. High with graduation
4. Community college/ technical school
5. University degree/bachelor's or equivalent
6. Graduate degree

Thinking about the person in your household with the highest level of schooling, what is the highest level of schooling they have completed?

1. Primary (0-6 years)
2. High school without graduation
3. High with graduation
4. Community college/ technical school
5. University degree/bachelor's or equivalent
6. Graduate degree

What is your Parental education?

1. Primary (0-6 years)
2. High school without graduation
3. High with graduation
4. Community college/ technical school
5. Graduate degree

How will you best describe your current employment situation?

1. Unemployed not seeking work (student/homemaker etc)
2. Unemployed seeking work
3. Employed part time (< 30 hrs a week)
4. Employed full time (> 30 hrs a week)

How would you best describe your mother's current employment situation?

1. Unemployed not seeking work
2. Unemployed seeking work
3. Employed part time (< 30 hrs a week)

4. Employed full time (> 30 hrs a week)
<p>Q21 How would you best describe your father's current employment situation?</p> <ol style="list-style-type: none"> <li>1. Unemployed not seeking work</li> <li>2. Unemployed seeking work</li> <li>3. Employed part time (&lt; 30 hrs a week)</li> <li>4. Employed full time (&gt; 30 hrs a week)</li> </ol>
<p>Do you currently have insurance that covers all or part of your dental expenses?</p> <ol style="list-style-type: none"> <li>1. Yes</li> <li>2. No</li> <li>3. Prefer Not to Answer</li> <li>4. Don't know</li> </ol>
<p>How often do you usually see a dental professional, such as a dentist, a dental hygienist?</p> <ol style="list-style-type: none"> <li>1. Once or more than once a year</li> <li>2. Less than once a year</li> <li>3. Only for emergency care</li> <li>4. Never</li> <li>5. Prefer Not to Answer</li> <li>6. Don't know</li> </ol>
<p>When was the last time you saw a dental professional, such as dentist or a dental hygienist/?</p> <ol style="list-style-type: none"> <li>1. Less than 1 year to 1 year ago</li> <li>2. More than 1 year to 2 years ago</li> <li>3. More than 2 years to 3 years ago</li> <li>4. More than 3 years to 4 years ago</li> <li>5. More than 4 years to 5 years ago</li> <li>6. More than 5 years ago</li> <li>7. Never</li> </ol>

8. RF

9. DK

How often do you brush your teeth?

1. Twice a day or more

2. Once a day

3. Less than once a day

4. Never

5. Prefer Not to Answer

6. Don't know

At the present time, do you smoke cigarettes frequently, occasionally or not at all?

1. Daily

2. Occasionally

3. Not at all

4. RF

5. DK

In the past 12 months:

Have you had any pain in your teeth or mouth?

1. Never

2. Hardly ever

3. Occasionally

4. Fairly often

5. Very often

8. RF

9. DK

Have you had any difficulty sleeping because of problems with your teeth and mouth? 1. Never

2. Hardly ever

3. Occasionally

4. Fairly often

5. Very often

8. RF

9. DK

Have you had any difficulty working because of problems with your teeth and mouth? 1. Never

2. Hardly ever

3. Occasionally

4. Fairly often

5. Very often

6. RF

7. DK

Have you had any difficulty chewing because of problems with your teeth and mouth? 1. Never

2. Hardly ever

3. Occasionally

4. Fairly often

5. Very often

6. RF

7. DK

Have you ever felt uncomfortable or embarrassed because of problems with your teeth and mouth?

1. Never
2. Hardly ever
3. Occasionally
4. Fairly often
5. Very often
6. RF
7. DK

The questions below are about your feelings and thought during the last month, in each question circle on how you thought.

1. In the last month, how often have you been upset because of something that happened unexpectedly?

0. Never
1. Almost never
2. Sometimes
3. Fairly often
4. Very often

2. In the last month, how often have you felt that you were unable to control the important things in your life?

0. Never
1. Almost never
2. Sometimes
3. Fairly often
4. Very often

3. In the last month, how often have you felt nervous and "stressed"?

0. Never
1. Almost never

2. Sometimes
3. Fairly often
4. Very often
4. In the last month, how often have you felt confident about your ability to handle your personal problems?
0. Never
1. Almost never
2. Sometimes
3. Fairly often
4. Very often
5. In the last month, how often have you felt that things were going your way?
0. Never
1. Almost never
2. Sometimes
3. Fairly often
4. Very often
6. In the last month, how often have you found that you could not cope with all the things that you had to do?
0. Never
1. Almost never
2. Sometimes
3. Fairly often
4. Very often
7. In the last month, how often have you been able to control irritations in your life?
0. Never

1. Almost never
2. Sometimes
3. Fairly often
4. Very often

8. In the last month, how often have you felt that you were on top of things?

0. Never
1. Almost never
2. Sometimes
3. Fairly often
4. Very often

9. In the last month, how often have you been angered because of things that happened that were outside of your control?

0. Never
1. Almost never
2. Sometimes
3. Fairly often
4. Very often

10. In the last month, how often have you felt difficulties piling up so high that you could not overcome them?

0. Never
1. Almost never
2. Sometimes
3. Fairly often
4. Very often

Appendix 7: Oral Examination Screen Form



**World Health Organization**  
**Oral Health Assessment Form**  
**for Adults, 2013**

Leave blank (1) <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	Year (4) (5) <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	Month <input type="text"/> <input type="text"/>	Day <input type="text"/> <input type="text"/>	Identification No. (10) (11) <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/>	Orig/Dupl (14) <input type="text"/> (15)	Examiner (16) <input type="text"/> <input type="text"/> (17)																																																																																																																	
<b>General information:</b>				<b>Sex</b> 1=M, 2=F <input type="text"/> (18) (19)	<b>Date of birth</b> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> <input type="text"/> (24) (25)	<b>Age in years</b> <input type="text"/> <input type="text"/> (26)																																																																																																																	
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<b>Ethnic group</b> (27) <input type="text"/> <input type="text"/> (28)		<b>Other group</b> (29) <input type="text"/> <input type="text"/> (30)		<b>Years in school</b> (31) <input type="text"/> <input type="text"/> (32)		<b>Occupation</b> <input type="text"/> (33)																																																																																																																	
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						3 = Filled, no caries																																																																																																																	
						4 = Missing due to caries																																																																																																																	
						5 = Missing for any another reason																																																																																																																	
						6 = Fissure sealant																																																																																																																	
						7 = Fixed dental prosthesis/crown abutment, veneer, implant																																																																																																																	
						8 = Unerupted																																																																																																																	
						9 = Not recorded																																																																																																																	
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Pocket (157)	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	(172)																																																																																																					





## World Health Organization Oral Health Assessment Form for Adults, 2013

<p><b>Loss of attachment</b></p> <p><b>Severity</b>                  0 = 0–3 mm                  1 = 4–5 mm    Cemento-enamel junction (CEJ) within black band                  2 = 6–8 mm    CEJ between upper limit of black band and 8.5 mm ring                  3 = 9–11 mm    CEJ between 8.5 mm and 11.5 mm ring                  4 = 12 mm or more    CEJ beyond 11.5 mm ring                  X = Excluded sextant                  9 = Not recorded</p> <p>* Not recorded under 15 years of age</p>	<p><b>Index teeth</b></p> <table style="margin: auto; border-collapse: collapse;"> <tr> <td></td> <td style="text-align: center;">17/16</td> <td style="text-align: center;">11</td> <td style="text-align: center;">26/27</td> <td></td> </tr> <tr> <td style="text-align: center;">(173)</td> <td style="border: 1px solid black; width: 20px; height: 20px;"></td> <td style="border: 1px solid black; width: 20px; height: 20px;"></td> <td style="border: 1px solid black; width: 20px; height: 20px;"></td> <td style="text-align: center;">(175)</td> </tr> <tr> <td style="text-align: center;">(176)</td> <td style="border: 1px solid black; width: 20px; height: 20px;"></td> <td style="border: 1px solid black; width: 20px; height: 20px;"></td> <td style="border: 1px solid black; width: 20px; height: 20px;"></td> <td style="text-align: center;">(178)</td> </tr> <tr> <td></td> <td style="text-align: center;">47/46</td> <td style="text-align: center;">31</td> <td style="text-align: center;">36/37</td> <td></td> </tr> </table>		17/16	11	26/27		(173)				(175)	(176)				(178)		47/46	31	36/37		<p><b>Enamel fluorosis</b> <input style="width: 20px; height: 20px;" type="text"/> (179)</p> <p><b>Severity</b>                  0 = Normal                  1 = Questionable                  2 = Very mild                  3 = Mild                  4 = Moderate                  5 = Severe                  8 = Excluded (crown, restoration, "bracket")                  9 = Not recorded (unerupted tooth)</p>
	17/16	11	26/27																			
(173)				(175)																		
(176)				(178)																		
	47/46	31	36/37																			
<p><b>Dental erosion</b></p> <p><b>Severity</b> <input style="width: 20px; height: 20px;" type="text"/> (180)</p> <p>0 = No sign of erosion                  1 = Enamel lesion                  2 = Dentinal lesion                  3 = Pulp involvement</p> <p><b>Number of teeth affected</b>                  (181) <input style="width: 20px; height: 20px;" type="text"/> <input style="width: 20px; height: 20px;" type="text"/> (182)</p>	<p><b>Dental trauma</b></p> <p><b>Status</b> <input style="width: 20px; height: 20px;" type="text"/> (183)</p> <p>0 = No sign of injury                  1 = Treated injury                  2 = Enamel fracture only                  3 = Enamel and dentine fracture                  4 = Pulp involvement                  5 = Missing tooth due to trauma                  6 = Other damage                  9 = Excluded tooth</p>	<p><b>Number of teeth affected</b>                  (184) <input style="width: 20px; height: 20px;" type="text"/> <input style="width: 20px; height: 20px;" type="text"/> (185)</p>																				
<p><b>Oral mucosal lesions</b></p> <p><input style="width: 20px; height: 20px;" type="text"/> (186)  <input style="width: 20px; height: 20px;" type="text"/> (187)  <input style="width: 20px; height: 20px;" type="text"/> (188)</p> <p><b>Condition</b>                  0 = No abnormal condition                  1 = Malignant tumour (oral cancer)                  2 = Leukoplakia                  3 = Lichen planus                  4 = Ulceration (aphthous, herpetic, traumatic)                  5 = Acute necrotizing ulcerative gingivitis (ANUG)                  6 = Candidiasis                  7 = Abscess                  8 = Other condition (specify if possible)                  9 = Not recorded</p>	<p><input style="width: 20px; height: 20px;" type="text"/> (189)  <input style="width: 20px; height: 20px;" type="text"/> (190)  <input style="width: 20px; height: 20px;" type="text"/> (191)</p> <p><b>Location</b>                  0 = Vermillion border                  1 = Commissures                  2 = Lips                  3 = Sulci                  4 = Buccal mucosa                  5 = Floor of the mouth                  6 = Tongue                  7 = Hard and/or soft palate                  8 = Alveolar ridges/gingiva                  9 = Not recorded</p>	<p><b>Denture(s)</b></p> <p style="text-align: center;"><b>Upper</b>      <b>Lower</b></p> <p style="text-align: center;"><input style="width: 20px; height: 20px;" type="text"/> (192)    <input style="width: 20px; height: 20px;" type="text"/> (193)</p> <p><b>Status</b>                  0 = No denture                  1 = Partial denture                  2 = Complete denture                  9 = Not recorded</p>																				
<p><b>Intervention urgency</b> <input style="width: 20px; height: 20px;" type="text"/> (194)</p> <p>0 = No treatment needed                  1 = Preventive or routine treatment needed                  2 = Prompt treatment (including scaling) needed                  3 = Immediate (urgent) treatment needed due to pain or infection of dental and/or oral origin                  4 = Referred for comprehensive evaluation or medical/dental treatment (systemic condition)</p>																						

## Appendix 8: Health Science Research Ethics Board Approvals



**Date:** 20 September 2021

**To:** Dr Sharat Pani

**Project ID:** 119492

**Study Title:** Long-term stress, geographic indicators, and sociodemographic variables as predictors of dental treatment outcomes among adolescents transitioning into adulthood

**Application Type:** HSREB Initial Application

**Review Type:** Delegated

**Meeting Date / Full Board Reporting Date:** 05/October/2021

**Date Approval Issued:** 20/Sep/2021

**REB Approval Expiry Date:** 20/Sep/2022

Dear Dr Sharat Pani

The Western University Health Science Research Ethics Board (HSREB) has reviewed and approved the above mentioned study as described in the WREM application form, as of the HSREB Initial Approval Date noted above. This research study is to be conducted by the investigator noted above. **All other required institutional approvals and mandated training must also be obtained prior to the conduct of the study.**

**Documents Approved:**

Document Name	Document Type	Document Date	Document Version
Advertisement for participant recruitment Research	Recruitment Materials	13/Aug/2021	1
Stress_and_Socioeconomic_Position_Questionnaire	Online Survey	09/Sep/2021	2
Research Proposal, clean version 2	Protocol	01/Sep/2021	2
Telephone_Script_clean version 3	Telephone Script	16/Sep/2021	3
Infomed Consent, Adults_clean version 5	Written Consent/Assent	16/Sep/2021	5
Infomed Consent, children, Parent_clean version-5	Written Consent/Assent	16/Sep/2021	5

**Documents Acknowledged:**

Document Name	Document Type	Document Date	Document Version
References	References	13/Aug/2021	1
Budget Naima	Study budget	16/Aug/2021	1
Flow chart-Clean version.	Flow Diagram	13/Aug/2021	2

No deviations from, or changes to, the protocol or WREM application should be initiated without prior written approval of an appropriate amendment from Western HSREB, except when necessary to eliminate immediate hazard(s) to study participants or when the change(s) involves only administrative or logistical aspects of the trial.

REB members involved in the research project do not participate in the review, discussion or decision.

The Western University HSREB operates in compliance with, and is constituted in accordance with, the requirements of the TriCouncil Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2); the International Conference on Harmonisation Good Clinical Practice Consolidated Guideline (ICH GCP); Part C, Division 5 of the Food and Drug Regulations; Part 4 of the Natural Health Products Regulations; Part 3 of the Medical Devices Regulations and the provisions of the Ontario Personal Health Information Protection Act (PHIPA 2004) and its applicable regulations. The HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 0000940.

Please do not hesitate to contact us if you have any questions. Sincerely,

Patricia Sargeant, Ethics Officer [REDACTED] behalf of Dr. Emma Duerden, HSREB Vice-Chair



**Date:** 22 September 2022

**To:** Dr Sharat Pani

**Project ID:** 119492

**Review Reference:** 2022-119492-71178

**Study Title:** Long-term stress, geographic indicators, and sociodemographic variables as predictors of dental treatment outcomes among adolescents transitioning into adulthood

**Application Type:** Continuing Ethics Review (CER) Form

**Review Type:** Delegated

**REB Meeting Date:** 11/Oct/2022

**Date Approval Issued:** 22/Sep/2022 09:53

**REB Approval Expiry Date:** 20/Sep/2023

**\*\*Lapse in REB approval 20/Sep/2022-22/Sep/2022\*\***

---

Dear Dr Sharat Pani,

The Western University Research Ethics Board has reviewed the application. This study, including all currently approved documents, has been re-approved until the expiry date noted above.

REB members involved in the research project do not participate in the review, discussion or decision.

Western University REB operates in compliance with, and is constituted in accordance with, the requirements of the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2); the International Conference on Harmonisation Good Clinical Practice Consolidated Guideline (ICH GCP); Part C, Division 5 of the Food and Drug Regulations; Part 4 of the Natural Health Products Regulations; Part 3 of the Medical Devices Regulations and the provisions of the Ontario Personal Health Information Protection Act (PHIPA 2004) and its applicable regulations. The REB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

Please do not hesitate to contact us if you have any questions.

**Electronically signed by:**

Patricia Sargeant, Ethics Officer [REDACTED] HSREB Chair 22/Sep/2022 09:53

**Reason:** I am approving this document

**Note:** This correspondence includes an electronic signature (validation and approval via an online system that is compliant with all regulations).



**Date:** 31 August 2023

**To:** Dr Sharat Pani

**Project ID:** 119492

**Review Reference:** 2023-119492-82738

**Study Title:** Long-term stress, geographic indicators, and sociodemographic variables as predictors of dental treatment outcomes among adolescents transitioning into adulthood

**Application Type:** Continuing Ethics Review (CER) Form

**Review Type:** Delegated

**Date Approval Issued:** 31/Aug/2023 16:22

**REB Approval Expiry Date:** 20/Sep/2024

---

Dear Dr Sharat Pani,

The Western University Research Ethics Board has reviewed the application. This study, including all currently approved documents, has been re-approved until the expiry date noted above.

REB members involved in the research project do not participate in the review, discussion or decision.

Western University REB operates in compliance with, and is constituted in accordance with, the requirements of the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2); the International Conference on Harmonisation Good Clinical Practice Consolidated Guideline (ICH GCP); Part C, Division 5 of the Food and Drug Regulations; Part 4 of the Natural Health Products Regulations; Part 3 of the Medical Devices Regulations and the provisions of the Ontario Personal Health Information Protection Act (PHIPA 2004) and its applicable regulations. The REB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

Please do not hesitate to contact us if you have any questions.

**Electronically signed by:**

Mr. Joshua Hatherley, Ethics Coordinator on behalf of [REDACTED] HSREB Chair 31/Aug/2023 16:22

**Reason:** I am approving this document

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Publication Title	Community dentistry and oral epidemiology	Publication Type	Journal
Article Title	The common risk factor approach: a rational basis for promoting oral health.	Start Page	399
Date	01/01/1973	End Page	406
Language	English	Issue	6
Country	Denmark	Volume	28
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#### NEW WORK DETAILS

Title	Long term stress, geographic indicators, and sociodemographic variables as predictors of dental treatment outcomes in adolescents and adulthood	Institution Name	Schulich School of Medicine & Dentistry, University of Western Ontario, London, ON, Canada
Instructor Name	Naïma Abouseta	Expected Presentation Date	2023-12-10

#### ADDITIONAL DETAILS

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#### REQUESTED CONTENT DETAILS

Title, Description or Numeric Reference of the Portion(s)	Figure 7: The Common Risk Factor Approach: Risk Factors common to a number of chronic diseases	Title of the Article/Chapter the Portion Is From	The common risk factor approach: a rational basis for promoting oral health.
Editor of Portion(s)	[REDACTED]	Author of Portion(s)	Sheiham, Aubrey; Watt, Richard Geddie
Volume / Edition	28	Publication Date of Portion	2002-01-04
Page or Page Range of Portion	399-406		

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ISSN	0140-6736	Portion	Chart/graph/table/figure

## LICENSED CONTENT

Publication Title	The Lancet	Rightsholder	Elsevier Science & Technology Journals
Article Title	Oral diseases: a global public health challenge.	Publication Type	Journal
Author / Editor	Wakley, Thomas	Start Page	249
Date	01/01/1823	End Page	260
Language	English	Issue	10194
Country	United Kingdom of Great Britain and Northern Ireland	Volume	394

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## NEW WORK DETAILS

Title	Relationships among long-term stress, geographic indicators and sociodemographic variables as predictors of dental treatment outcomes among adolescent and young adult	Institution Name	Western university
Instructor Name	Naïma Abouseta	Expected Presentation Date	2024-02-05

## ADDITIONAL DETAILS

Order Reference Number	N/A	The Requesting Person / Organization to Appear on the License	Naïma Abouseta
------------------------	-----	---	----------------

## REQUESTED CONTENT DETAILS

Title, Description or Numeric Reference of the Portion(s)	Figure 3: Social and commercial determinants of oral diseases	Title of the Article / Chapter the Portion is From	Oral diseases: a global public health challenge.
Editor of Portion(s)		Author of Portion(s)	Peres, Marco A; Macpherson, Lorna M B; Weyant, Robert J; Daly, Blánaid; Venturelli, Renate; Mathur, Manu R; Listi, Stefan; Celeste, Roger Keller; Guarnizo-Herreño, Carol C; Keams, Cristin; Benzian, Habib; Allison, Paul; Watt, Richard G
Volume / Edition	394	Issue, if Republishing an Article From a Serial	10194
Page or Page Range of Portion	249-260	Publication Date of Portion	2019-07-20

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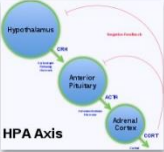
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Article Title	Relationships among cortisol, perceived stress, and dental caries experience in adolescents and young adults	Publication Type	e-Journal
Author / Editor	European Organization for Caries Research	Start Page	1
Date	01/01/1967	End Page	10
Language	English	URL	<a href="http://www.karger.com/Journal/Home/22...">http://www.karger.com/Journal/Home/22...</a>
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Lifetime Unit Quantity	Up to 499	Currency	CAD
<b>NEW WORK DETAILS</b>			
Title	Stress, geographic and sociodemographic factors, and oral health outcomes in adolescents and young adults	Institution Name	Western University
Instructor Name	Dr. Sharat Pani	Expected Presentation Date	2024-07-15
<b>ADDITIONAL DETAILS</b>			
Order Reference Number	N/A	The Requesting Person / Organization to Appear on the License	Naima Abouseita
<b>REQUESTED CONTENT DETAILS</b>			
Title, Description or Numeric Reference of the Portion(s)	Relationships among Cortisol, Perceived Stress, and Dental Caries Experience in Adolescents and Young Adults	Title of the Article / Chapter the Portion Is From	Relationships among cortisol, perceived stress, and dental caries experience in adolescents and young adults
Editor of Portion(s)	N/A	Author of Portion(s)	Abouseita, Naima; Gomaa, Noha; Tassi, Ali; Elzagallaai, Abdelbaset A.; Reeder, Michael J.; Dixon, S. Jeffrey; Pani, Sharat C.
Volume / Edition	24	Issue, if Republishing an Article From a Serial	N/A
Page or Page Range of Portion	1-10	Publication Date of Portion	2024-04-24

## Curriculum Vitae

**Name:** Naima Mohamed Abouseta

**Post-secondary Education and Degrees:** The University of Tripoli, Faculty of Dentistry, Tripoli, Libya  
1993-1998 B.A.

The University of Newcastle Upon Tyne  
Newcastle Upon Tyne, UK  
2003-2005 M.A.

The University of Western Ontario  
London, Ontario, Canada  
2020-2024 Ph.D.

**Honours and Awards:** M.A. & Ph.D. scholarships from Ministry of Higher Education, Tripoli, Libya  
2003-2005, 2020-2024

The University of Western Ontario, Schulich Dentistry Research Day Award. \$250.  
2022

Inspiring Minds Showcase, highlighted by Western.  
The University of Western Ontario  
2023

**Related Work Experience** Research assistance, COVID-19 experience in Canadian dental schools.  
The University of Western Ontario  
2021-2022 demeanor,

Lecturer in the Pediatric Division of the Orthodontics Department  
The University of Tripoli, Faculty of Dentistry  
2008-2017

Instructor and clinical supervisor  
The University of Tripoli, Faculty of Dentistry  
2008-2017

Work experience as a dentist in Sukra Dental Clinic and Sant James's hospital  
2000-2002  
Tripoli, Libya

Undergraduate Teaching Assistant  
The University of Tripoli, Faculty of Dentistry  
2000-2002

**Publications:**

1. Abouseta N, Gomaa N, Dixon SJ, Pani SC. Neighborhood-Level Inequalities in Dental Care of Adolescents and Young Adults in Southwestern Ontario. *Children*. 2022; 9(2):183.
2. Abouseta N, Gomaa N, Tassi A, Elzagallaai AA, Rieder MJ, Dixon SJ, Pani SC. Relationships among Cortisol, Perceived Stress, and Dental Caries Experience in Adolescents and Young Adults. *Caries Research*. 2024 Apr 24:1-10. Online ahead of print.
3. Abulwefa A, Eldaissi A, Abouseta N. *Pedodontics examination review*. 1<sup>st</sup> edition, Libya (Tripoli): Dar Alhikma. 2017.
4. Abulwefa A, Manhal S, Abouseta N, Singh C. 2012. Supernumerary Teeth: Characteristics and Management. *Libyan Medical Journal*. 2012; 3:1-14.

**Participation in Conferences:**

1. Abouseta, et al. (2021) “Neighborhood-Level Inequalities in Dental Care of Adolescents and Young Adults in Southwestern Ontario.” 99th International Association for Dental Research (IADR) Meeting. Alexandria, VA, USA. Virtual International poster.
2. Abouseta, et al. (2021) “Neighborhood-Level Inequalities in Dental Care of Adolescents and Young Adults in Southwestern Ontario.” London Health Research Day – Virtual. Virtual poster.
3. Abouseta, et al (2022) “Neighborhood-Level Inequalities in Dental Care of Adolescents and Young Adults in Southwestern Ontario.” Schulich Dentistry Research Day, 2022. Virtual oral presentation.
4. Abouseta, et al (2023) “The relationship between cortisol, perceived stress, and dental caries experience in adolescents and young adults.” DART Student Dental Research Symposium. Schulich School of Medicine & Dentistry, Western University. Local-Poster.
5. Abouseta, et al (2023) “The relationship between cortisol, perceived stress, and dental caries experience in adolescents and young adults.” London Health Research Day, 2023. In person poster.
6. Abouseta, et al (2024) “The relationship between cortisol, perceived stress, and dental caries experience in adolescents and young adults.” 1st CADR NSRG Students Virtual Research Competition. Virtual presentation.