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The role of the oral microbiota related to periodontal diseases in anxiety, depression and and stress

A Project Submitted to

The College of Dentistry, University of Baghdad, Department of Periodontics in Partial Fulfilment for the Bachelor of Dental Surgery

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بسم الله الرحمن رحيم

قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْتَنَا
إِنَّكَ أَنتَ الْعَلِيمُ الْحَكِيمُ

صدق الله العظيم

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Certification of the Supervisor

I certify that this project entitled **''The Role of the Oral Microbiota Related to Periodontal Diseases in Anxiety , Depression and and Stress''** was prepared by **(Afnan Rashad Hajwal)** under my supervision at the College of Dentistry / University of Baghdad in partial fulfillment of the graduation requirements for the Bachelor degree in dentistry.

Signature
Assist. Prof. Nada Kadhim Imran
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The supervisor

Dedication

We dedicate this project to God Almighty our creator, our strong pillar, our source of inspiration, wisdom, knowledge and understanding. He has been the source of our strength and on His wings only we have soared

And with all the love and respect, we dedicate this project to our lovely mother, father, brother, sister and our friends for their great support and for always believing in us.

To our all friends and colleagues.

Finally to our supervisor who encourages us to keep go.

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Thank everyone who helped us in the completion of the search for scientific truth

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List of abbreviations

Abbreviation	Scientific name
TNF-α	Tumor necrosis factor alpha
IL-1β	Interleukin-1 beta
IL-6	Interleukin-6
IFN-γ	Interferon gamma
IL-2	Interleukin-2
IL-4	Interleukin-4
SES	Socioeconomic status
HIV	Human immunodeficiency virus
AIDS	Acquired immunodeficiency syndrome
AAR	Ask, Advise, Refer
AAP	American Academy of Periodontology
HbA1C	Hemoglobin A1c
DM	Diabetes mellitus
HPA	Hypothalamic-pituitary-adrenal
LPS	Lipopolysaccharides

MAMPs	Microbe-associated molecular patterns
PAMPS	Pathogen-associated molecular patterns
CNS	Central nervous system
CRP	C-reactive protein
SRP	Scaling and root planing
BBB	Blood–brain barrier
IL-1	Interleukin-1
PD	Periodental Disease
p75NTR	P75 neurotrophin receptor
TLR4	Toll-like receptor4
BDNF	Brain Derived Neurotrophic Factor

Abstract

Background: periodontal diseases encompass a group of infectious and inflammatory diseases that affect the periodontium. Among them, periodontitis is defined as a chronic, multi-bacterial infection that elicits low-grade systemic inflammation via the release of proinflammatory cytokines, as well as local invasion and long-distance translocation of periodontal pathogens. Anxiety and depression are types of mood disorders. Among them, depression causes feelings of sadness, hopelessness, and reduced energy, while anxiety creates feelings of nervousness, worry, or dread. Stress can be defined as a state of worry or mental tension caused by a difficult situation. Stress is a natural human response that prompts us to address challenges and threats in our lives. Periodontal pathogens have been implicated in the etiology and pathophysiology of neuropsychiatric disorders (such as depression and schizophrenia), especially as dysregulation of the immune system also plays an integral role in the etiology and pathophysiology of these disorders.

Aim of the study:to assess the role of the oral microbiota related to periodontal diseases in anxiety, depression and and stress.

Conclusion: epidemiological association between anxiety, depression ,stress and diseases with altered oral microbiota such as periodontitis has been found that can be induced and followed by subsequent models to induce anxiety- and/or depression like endophenotypes. Periodontal bacteria have several mechanisms whereby they can transduce peripheral inflammation into neuroinflammation, and influence central nervous system functioning and behavior . Mental health disorders and oral health may be associated via changes to the oral microbiome , involving increased pro-inflammatory communication and cortisol in saliva.

Keywords: oral microbiota, anxiety disorders, periodontitis,stress, derpression disorders

Aim of the study

to assess the role of the oral microbiota related to periodontal diseases in anxiety, depression and stress.

Chapter one

Review of literature

Chapter One

1.1. Periodontal disease:

1.1.1. Introduction:

Periodontal diseases are one of the main causes of tooth loss and the second most common oral disease after dental caries[(1)] and can be divided into gingivitis and periodontitis. Gingivitis is an inflammatory condition of the gingival tissue, most commonly caused by bacterial infection, there is no attachment loss and migration of the junctional epithelium. The condition is restricted to the soft-tissue area of the gingival epithelium and connective tissue, clinically, the gingival tissues are characterized by swelling, redness, tenderness, a shiny surface, and bleeding upon gentle probing. Gingivitis seldom generates spontaneous bleeding and is commonly painless, therefore many patients do not recognize the disease and fail to seek attention[(2)]. Periodontitis is one of the most common ailments affecting the teeth, leading to the destruction of the supporting and surrounding tooth structure " Periodontitis is originally a disease originating from the gingival tissue which if left untreated results in penetration of inflammation to the deeper tissues, altering the bone homeostasis and causing tooth loss"[(3)].

1.1.2. Pathogenesis of periodontal disease:

Periodontitis is a chronic multifactorial disease characterized by an inflammation of the periodontal tissue mediated by the host, which is associated with dysbiotic plaque biofilms, resulting in the progressive destruction of the tooth supporting apparatus and loss of periodontal attachment. The bacterial biofilm formation initiates gingival inflammation; however, periodontitis initiation and progression depend on dysbiotic ecological changes in the microbiome in response to nutrients from gingival inflammatory and tissue breakdown

products and anti-bacterial mechanisms that attempt to contain the microbial challenge within the gingival sulcus area once inflammation has initiated. This leads to the activation of several key molecular pathways, which ultimately activate host-derived proteinases that enable loss of marginal periodontal ligament fibers, apical migration of the junctional epithelium, and allows apical spread of the bacterial biofilm along the root surface. Therefore, the disease of periodontitis is characterized by three factors[4]:

• The loss of periodontal-tissue support, manifested through clinical attachment loss (CAL) and radiographically assessed alveolar bone loss.

• The presence of periodontal pocketing.

• Gingival bleeding.

Perturbation in the composition and function of the indigenous oral microbiome may determine an alteration of the symbiotic interaction between the oral microbial community and the host with consequences for the oral and general health of the individual.The alteration of this finely-tuned equilibrium between host and hosted microbes (dysbiosis), allows pathogenic bacteria to manifest their disease-promoting potential and determinate pathological conditions[5]. Our drastically increased understanding of the dynamic interactions between the various microbial and host factors has led to a new microbial model of periodontal pathogenesis, according to which the pathogenic process that drives periodontal tissue destruction is not related to a limited number of periodontopathogenic species but is the outcome of a synergic action of dysbiotic microbial communities. Dysbiosis seems to be globally associated with an increase in microbial diversity since the perturbation of the microbial environment allows certain indigenous species to expand and provides ideal conditions for the growth advantages of opportunistic microbes[6]. The host immune-inflammatory response in periodontitis is initially characterized by a physiological acute inflammation reaction (gingivitis) to supragingival and subgingival plaque, sustained by the cell of the innate immune system, including resident cells (epithelial cell and fibroblast),

phagocytic cells (macrophages and neutrophils), complement proteins and neuropeptides[7]. In this phase, cytokines produced by the residential cell population such as tumor necrosis factor (TNF)-α, interleukin 1beta (IL-1β), and (IL-6) have the main function to stimulate cells migration to sites of infection and enhance the expression of adhesion molecules for neutrophils on the internal vessel surfaces and increase the synthesis of other proinflammatory cytokines. The elimination of plaque leads to the progressive resolution of the inflammation and the restoration of individual homeostasis. Plaque persistence results in the activation of acquired immunity, this event occurs through antigen processing and presentation by lymphocytes, macrophages, and dendritic cells and regulated by adaptive-immunity cytokine, including interferon gamma (IFN-γ) and (IL-2) and (IL-4) [8].

1.1.3. Risk factors of periodontal diseases:

1.1.3.1. Introduction:

The risk is represented by the likelihood that a person develops a disease within a certain period of time. Risk factors sum up the characteristics of individuals who place them in the high risk category for developing a disease. The risk factor is defined as "any characteristic, behaviour or exposure associated with a certain disease. A risk factor is an aspect of personal behavior, lifestyle, exposure to environmental factors, or an innate or inherited feature that based on epidemiological evidence and is associated with a condition that influences health. Risk factors act in a very personal way, where the same set of risk factors that cause increased susceptibility to periodontitis to a person may have no effect on another person[9].

1.1.3.2. Typts of risk factors:

Risk factors are assocciated with periodental disease but not necessarily cause of periodental disease[10].There are Clinical observations and epidemiologic studies suggest that stress, depression and anxiety are potential factors that may affect periodontal disease.[11]

There are multiple types of risk factors can be divided into modifiable risk factors and non-modifiable risk factors as seen in table(1-1).

Table(1-1): modifiable risk factors and non-modifiable risk factors of periodental disease:

Modifiable risk factors	**Non-modifiable risk factors**
A-Specific microorganisms	A- Hematological Disorders
B-Diabetes mellitus	B- Genetic factors
C- Psychological factors	C- Aging
D- Obesity	D- gender
E- Socioeconomic status (SES)	E- Ethnicity
F- Pregnancy	F- HIV \ AIDS
G- Medications	G-Osteoporosis

A- Depressive disorders:

Are characterized by sadness, loss of interest and pleasure, feelings of guilt or , disturbed sleep or appetite, feelings of tiredness, and poor concentration. They can be persistent and recur, leading to problems functioning at work or coping with daily life.[(12)] Data of study done in 2015 indicate the prevalence of depression to be 4.4%, and more prevalent in females,this is roughly 322 million people worldwide.[(12)] Depression might

affect progression of periodontal infections in patients susceptible to periodontitis [13] and might be associated with a worse treatment outcome through a delay of wound healing by chronic dysregulated hypothalamic-pituitary-adrenal (HPA) axis and further determines cortisol and adrenal disturbances, as well as immune dysfunction and excessive secretion of proinflammatory cytokines.[14] Changes in health-related behaviors, such as oral hygiene, smoking, diet, alcohol consumption that occurs in depressed patients can also be related to the occurrence of periodontal disease.[15]Moreover, the antidepressive medication may also lead to xerostomia alterations in gingival circulation and changes in saliva composition that might result in an exacerbation of periodontitis.[16]

B- Anxiety disorders:

Are characterized by feelings of anxiety and fear. They can be broadly described as a group of disorders in which anxiety is induced in situations that may or may not be well defined. These situations often produce a feeling of strong avoidance, or they are endured with apprehension. Examples of physical symptoms include palpitations, shortness of breath, or feeling faint, and they are often accompanied with irrational thoughts, such as fears of dying, losing control, or going mad, anxiety and depression are often coexistent conditions.[12] The prevalence of anxiety disorders is around 3.6% and again more common in females, equating to 264 million people worldwide. Interestingly, prevalence does not vary considerably between age groups.[17] There are multiple studies have examined the negative impact of anxiety on our body and especially on our immune system, supporting its association with various inflammatory diseases such as periodontitis. Also, anxiety, in addition to its immunosuppressive properties, adversely affects the lifestyle of patients, ignoring their oral health with consequent adverse effects on gum health. **(Di Ve- nere et al.2015)[18]** in their study conclude that substance abuse by people with severe anxiety, such as tobacco and alcohol, plays a role in reducing salivary secretion and increasing the risk of periodontitis.

C-Stress:

Stress describes the effects of psychosocial and environmental factors on physical and/or mental well-being. These psychosocial and environmental factors are known as stressors, and they challenge the organism's normal homeostatic mechanisms, thereby eliciting a set of physiological reactions. In situations where stress is acute, the stress response initiates the host's immune system for subsequent challenge. By contrast, chronic stress may result in long-term inflammatory processes that can contribute to disease either locally or systemically, such as diabetes mellitus, cardiovascular disease, and periodontitis.[19] Patients with inadequate stress behavior stratgies (defensive coping) are at greater risk for severe periodontal disease. [20] Stress is associated with poor oral hygiene, increased glucocorticoid secretion that can depress immune function, increased insulin resistance, and potentially increased risk of periodontitis . The individuals, belonging to all age groups, communities, categories and backgrounds experience stress in their personal as well as professional lives. Stress has various positive aspects as well, but normally, when it is experienced in a major form, it has unfavorable effects upon the overall quality of lives of the individuals. The individuals need to pay attention towards promoting good health and well-being. When they will possess good health, they will be able to benefit to a major extent in generating the desired outcomes and sustaining one's living conditions in an adequate manner. When the individuals are conducting research on types of stress, they found different types of stress like **[Acute Stress, Episodic Acute Stress, Chronic Stress, Emotional Stress, Physical Stress, Psychological Stress, Psycho-social Stress].**[21]

1.2. Oral microbiota:

1.2.1. Introduction:

Oral microbiota organized in biofilms, defined as matrix embedded microbial populations, adherent to each other and/or to surfaces or interfaces (teeth, restorations, or soft tissues). A perturbation in these biofilms may lead to dysbiosis (where the equilibrium that usually exists between different bacteria is disturbed), which could influence the immune system to promote inflammation and ultimately drive the development of different diseases, such as dental caries, periodontal diseases, or peri-implant diseases. One of the possible mechanisms whereby the microbiota could contribute to neuroinflammation is via circulating endotoxins, i.e., lipopolysaccharides (LPS), which are part of the outer membrane of Gram-negative bacteria, or other microbe -associated molecular patterns or pathogen-associated molecular patterns (MAMPs or PAMPs). In the case of LPS, toxicity is associated with the lipid component and immunogenicity is associated with the polysaccharide components, eliciting a variety of inflammatory responses. Endotoxins can enter the circulation more readily through compromised internal barriers, such as the oral and intestinal mucosa, thereby allowing toxins to spread systemically, resulting in an inflammatory cascade in the central nervous system (CNS).Indeed, leaky gut (due to compromised tight junctions in the intestinal lining) and subsequent bacterial translocation have been reported in depression and schizophrenia studies[(22)]. In this same line, the presence of a "leaky mouth" (due to the widening of the intercellular spaces between the epithelial cells and the rupture of the epithelium on the periodontal pocket in patients with periodontitis), has been proposed as being able to lead to the translocation of bacteria/inflammatory mediators across the oral mucosa into systemic circulation, and distant organs and tissues. There are different niches in the oral cavity due to the different environmental conditions or nutrient availability, for example, the subgingival or the supragingival niches, niches in dental fissures, tonsils or tongue,

all bathed by saliva. Although while dealing with the study of oral microbiome it would be ideal to focus on each niche independently, there are also some publications considering the whole mouth as just one niche. In this review we are presenting both approaches depending on the information reported in the original studies, focusing on the subgingival niche whenever possible, as it is the one related to periodontal diseases. However, for those cases in which there was no direct evidence about the specific role of subgingival [(23)].

1.2.2. Effects of stress, anxiety and depression on oral microbiota

The pathways by which stress, anxiety and depression are associated with oral microbial changes may involve behavioral, Immunological and/or neuroendocrine mechanisms models that alter salivary composition.

A-Behavioral models: suggest that the clinical impairment of living with depression and anxiety can interfere with the maintenance of dental care routines: stress,anxiety and dental depression are associated with lower rates of tooth brushing and decreased utilization of services [(24)].The co-occurrence of mental illness and oral disease, involving pathogenicbacteria that may develop in response to poorer oral hygiene routines in those with anxiety and depression.

B-A preliminary body of literature also suggests stress,anxity and depression contribute to oral disease via **neuroendocrine mechanisms**, specifically stress hormone secretion into the oral cavity such as (cortisol). Normative (Hypothalamic-pituitary-adrenal) axis activity prepares the body for physiological and psychological stressors via the release of stress hormones (e.g., cortisol), where elevated levels of basal cortisol are associated with stress ;therefore, high levels of cortisol secreted into the mouth, via the salivary glands, may impact oral health and the oral microbiome in these mental health disorders[(25)]. Consistently, increased levels of cortisol in the gingival crevicular fluid of women with depression has

been reported relative to controls, which was accompanied by higher level of dental plaque and gingival inflammation .

C-Immunological models: postulate that internalizing disorders are characterized by dysregulated neuroimmune pathways, involving increased inflammatory signaling between the periphery and the brain, occurring as early as in adolescence. Sustained immune system activation may induce elevated synthesis of pro-inflammatory molecules that dysregulate the (HPA) axis [(26)]. Poor oral hygiene can be a by-product of mental illness, inflammation in oral cavity precedes anxiety and depression in adults, and stress hormones change salivary components and, thus, microbial composition.

1.2.2. Influences of periodontal microbiota on the central nervous system and behavior :

Periodontal bacteria and bacterial molecules have several possible means whereby they can impact the CNS. They can directly invade the brain via the bloodstream or via cranial nerves. Lipopolysaccharides can lead to the deterioration of the blood–brain barrier (BBB) and increase its permeability, thereby providing circulating periodontal bacteria/bacterial molecules the opportunity to penetrate into the CNS through a compromised BBB [(27)]. Other points of entry include the circumventricular organs and choroid plexus (which lack a contiguous BBB), as well as the olfactory and trigeminal nerves [(28)]. Periodontal bacteria and/or bacterial molecules can also communicate with brain-resident microglia through the leptomeninges. Furthermore, periodontitis induces systemic inflammation and proinflammatory cytokines can activate endothelial cells that express receptors for TNF-α and IL-1, which, in turn, signal to the perivascular macrophages located immediately adjacent to cerebral endothelial cells, these perivascular macrophages subsequently communicate with microglia and thus lead to microglial activation and subsequent neuroinflammation [(29)]. Periodontal bacteria therefore have several mechanisms whereby they can transduce peripheral inflammation, including periodontitis, into

neuroinflammation, and thereby influence CNS functioning and behavior as shown in **(Figure 1-1).**

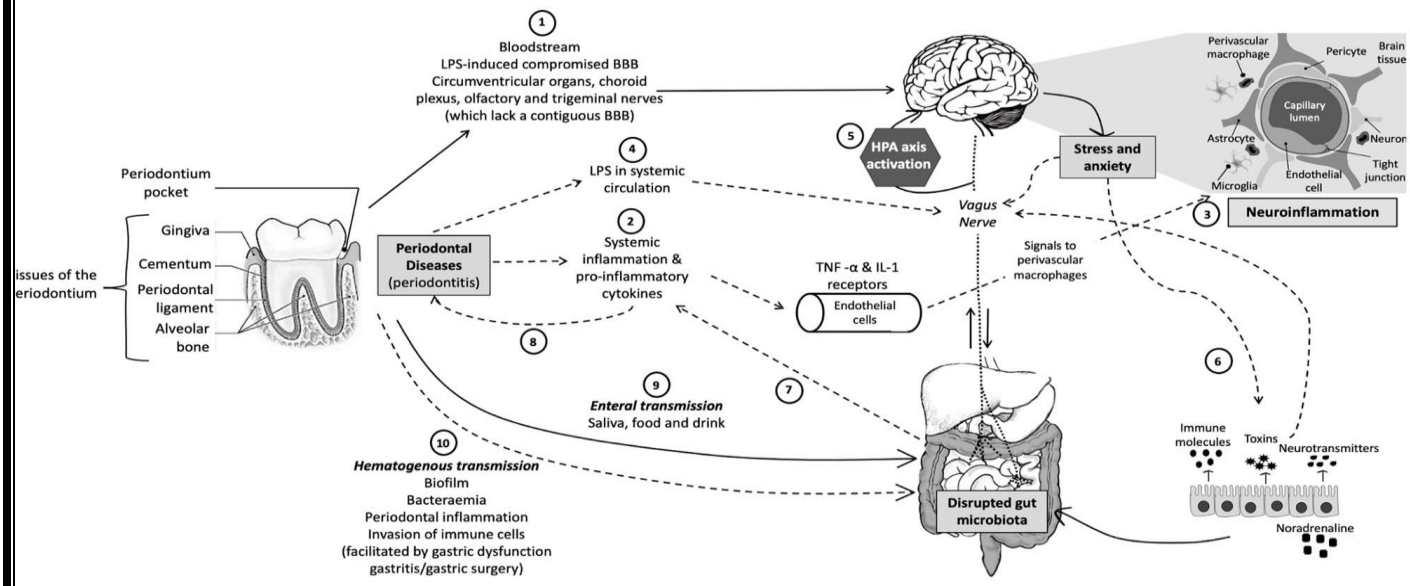

Figure (1-1):Schematic represent action of the oral-gut-brain axis

1.2.3. Clinical investigations of effects of oral microbiota composition on depression, anxiety and stress

At first glance it might seem that poor mental health is the driver in the relationship between oral and mental health, a large-scale (n > 60 000), longitudinal (10-year follow-up) study detected that there was a higher incidence of subsequent development of depression in individuals in the periodontitis group compared to the non-periodontitis group. This finding provides evidence that periodontitis would be a risk factor for the later development of major depression[(30)]; therefore, it is possible that periodontitis may be a susceptibility factor in mental health conditions. The human oral bacterial community might hold some clues; great advances have been made to comprehensively catalog the diversity of bacteria and archaea in the human mouth and to identify associations between specific taxa and health and disease states as seen in table (1-2).

Table (1-2): Summary of taxa implicated in periodontal diagnoses as well as anxiety disorders, depressive disorders, and stress.

Taxa	Results
Prevotella	*Prevotella intermedia*, and *Prevotella nigrescens* was correlated with diseased periodontal tissues
	Negatively associated with distress and negatively associated with inflammatory markers and part of a consortium of taxa that could accurately predict distress and inflammation (saliva samples)
	Decreased in schizophrenia and mania cohorts (oropharyngeal samples)
Neisseria	Decreased in schizophrenia and mania cohorts (oropharyngeal samples)
	Neisseriales order was positively associated with CRP; *Neisseriaceae* family was positively associated with CRP and cortisol (saliva samples)
Fusobacterium	*Fusobacterium nucleatum* was correlated with diseased periodontal tissues
	Positively correlated with depression (saliva samples)
Leptotrichia	Associated with persistent generalized periodontal disease
	Positively correlated with depression and cortisol levels (saliva samples)
	Positively associated with distress and host inflammation (saliva samples)
Streptococcus	Higher proportions in patients successfully treated using active periodontal treatment (saliva samples)
	A *Streptococcus* taxon was associated with cortisol, anxiety and depression
	Streptococcus taxa could accurately predict distress
Selenomonas	Associated with persistent generalized periodontal disease
	Implicated to be involved in the pathogenesis of periodontitis
	A *Streptococcus* taxon was associated with cortisol, anxiety and depression

CRP, C-reactive protein.

The authors reported a diurnal pattern in the relative abundances of specific taxa over time: *Neisseria*, *Prevotella,* and *Bacertoides* were strongly associated with waking samples and *Veillonellaceae*, *Ruminococcaceae,* and *Sphingomonas* were associated with later time points. The authors commented that it is possible that lower alpha diversity in saliva could be associated with better oral health, which is contradictory to what has been observed in the gut microbiota **(31),** and therefore requires further investigation.

Additionally, individuals with high levels of distress also displayed greater homogeneity in microbial community structure**(31)**. The relative abundances of *Leptotrichia*, *Bacteroidetes*, *Selenomonas*, and *Haemophilus* were positively associated with distress, while the relative abundance of *Prevotella* was negatively associated with distress.

The relative abundances of *Leptotrichia*, *Capnocytophaga*, *Treponema*, and *Bacteroidetes* were positively associated with host inflammation, whereas the relative abundances of *Aggregatibacter*, *Bifidobacterium*, *Prevotella*, and *Veillonella* were negatively associated with inflammatory markers.

About 22% of taxa were significantly associated with both distress and inflammation and included features from *Bacteroidetes* and *Leptotrichia* taxa[(32)].

Itis however important to note that most studies used saliva samples or supragingival samples when investigating the oral microbiota. However, the ideal area to focus on the context of periodontitis is the subgingival microbiota.

A study done in 2020 they investigated anxietylike behaviors in the acute and chronic phase of ligature-induced murine model of PD.The ligature-induced model of PD used in this study can be divided into two distinct phases: acute and chronic[(33)], while the acute phase (≤14 days) is characterized by significant bone loss, pronounced inflammation of the affected region and elevated gene expression of pro-inflammatory cytokines, the chronic phase (>14 days) shows no further progression of bone loss and a constant state of inflammation . Thus, the presence of inflammatory signs (e.g.,gingival growth due to edema, erythema, and areas of ulceration) are clear signs of installed PD and the intensity of the symptoms can determine the severity of the disease. A growing problem among patients with PD is the presence of associated anxiety traits that can lead to treatment interruption, reduced treatment efficacy and aggravation of the severity of the disease. [(34)]

Another study done in 2019 shown the incidence of periodontitis is positive correlated with depression.[(35)] In their work demonstrates that *Pg(which* is a Gram-negative anaerobic pathogen of periodontitis) plays a role in the pathogenesis of depression in mice. The underlying mechanism is that *Pg* activates astrocytes through *Pg*-LPS and downregulates neurotrophin receptor (p75NTR) in astrocytes in a Toll Like Receptor 4

(TLR4)-dependent manner, resulting in the inhibition of Brain-derived neurotrophic factor (BDNF) maturation, which leads to depression in mice.

There is one study focus on the context of periodontitis is the subgingival microbiota used subgingival samples to investigate whether there were associations between psychosocial factors scores, salivary cortisol levels , clinical periodontal parameters and microbiota in periodontitis patient and subgingival microbiota samples were collected in two pathological sites (PPD ≥ 5 mm) and one healthy site of diseased patients (*n* = 30) [before/after scaling and root planning (SRP)] and from one healthy site from control patients (*n* = 30) (samples collected before/after SRP). Although they did not detect a correlation between salivary cortisol and self-reported stress/anxiety, cortisol levels were positively associated with periodontal pocket depth. Furthermore, high levels of *Tannerella forsythia* were present in the periodontal pocket samples of all highly stressed patients compared to those with low stress levels and *A. actinomycetemcomitans* was only detected in the pockets of non-anxious patients .[(36)]

1.3. Conclusion:

An epidemiological association between anxiety, depression ,stress and diseases with altered oral microbiota such as periodontitis has been found that can be induced and followed by subsequent models to induce anxiety- and/or depression like endophenotypes. Periodontal bacteria have several mechanisms whereby they can transduce peripheral inflammation into neuroinflammation, and influence central nervous system functioning and behavior .Mental health disorders and oral health may be associated via changes to the oral microbiome , involving increased pro-inflammatory communication and cortisol in saliva.

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