Association of Single Nucleotide Gene Polymorphism at Interleukin-1β in Severe Chronic Periodontitis and Aggressive Periodontitis in a Group of Egyptians

Thesis

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Dedication

This thesis is dedicated to my parents for their endless love and for great support and help from my father; I really honored to be your son, my wife for support and help and to my lovely sons

List of Contents

List of Tables

List of Figures

List of Abbreviations

AA	**:**	Aggregatibacter Actinomycetemcomitans
CAL	**:**	Clinical attachment loss
CGD	**:**	Chronic granulomatous disease
CP	**:**	Chronic periodontitis
DNA	**:**	Deoxyribonucleic acid
EDS	**:**	Ehlers-Danlos syndrome
GAP	**:**	Generalized aggressive periodontitis
GI	**:**	Gingival index
IFN-γ	**:**	Interferon gamma
IL-1 α	**:**	Interleukin-1 alpha
IL-1	**:**	Interleukin-1
IL-10	**:**	Interleukin-10
IL-1RN	**:**	Interleukin-1 receptor antagonist
IL-1β	**:**	Interleukin-1beta
IL-4	**:**	Interleukin-4
IL-6	**:**	Interleukin-6
LAD	**:**	Leukocyte adhesion deficiency
LAP	**:**	Localized aggressive periodontitis

MMPs	**:**	Matrix metalloproteinases
PCR	**:**	Polymerase chain reaction technique
PG	**:**	Porphyromonas gingivalis
PMNs	**:**	Polymorphonuclear leukocyte
SMS	**:**	Singleton- Merten syndrome
SNP	**:**	Single nucleotide polymorphism
TNF-α	**:**	Tumor necrosis factor-alpha
VNTR	**:**	Variable number tandem repeat

INTRODUCTION AND REVIEW OF LITERATURE

Periodontal disease is a longstanding multifactorial disease which caused by bacterial plaque. Its pathogenesis and progression are affected by host defense mechanisms. Periodontitis is characterized by gingival inflammation associated with loss of supportive connective tissues and loss of alveolar bone. Clinical findings include increased probing depth, bleeding on probing and bone loss seen on radiographs. Progression of the disease will cause increased mobility and eventual tooth loss. **(*Page et al., 1997*)**

In order to improve the understanding of the etiology and pathology of the periodontal diseases, different classifications of periodontal diseases have been reported over the years. ***Armitage 1999*** classified periodontal diseases into 8 groups which are Gingivitis, Chronic periodontitis, Aggressive periodontitis, Periodontitis as a manifestation of systemic diseases, Necrotizing periodontal diseases, Abscesses of the periodontium, Periodontitis associated with endodontic lesions and Developmental or acquired deformities and conditions. **(*Armitage 1999*)**

Chronic periodontitis is the most common form of periodontitis. It is known as an infectious disease resulting in inflammation within the supporting tissues of the teeth with

progressive attachment and bone loss. Its onset may be at any age, but it is most common to occur in adults and the prevalence and severity of the disease increases with age. Supragingival and subgingival calculus is a frequent finding and the amounts of microbial deposits are consistent with the severity of periodontal tissue destruction with no marked familial aggregation reported. **(*Burt 2005*)**

Chronic periodontitis usually occurs as a slowly progressive condition with periods of remission and exacerbation with variable distribution of periodontal destruction and no usual pattern, it may affect a variable number of teeth and it has variable rates of progression. The disease can be further characterized by its extent and severity. Extent is the number of sites involved and can be described as localized and generalized. Extent can be characterized as localized if less than 30% of the sites are affected and generalized if more than 30% of the sites are affected. Severity can be described for the entire dentition or for individual teeth and sites. On a general basis, severity can be categorized on the basis of the amount of clinical attachment loss (CAL) into, slight 1–2 mm CAL, moderate 3–4 mm CAL, and severe 5 mm or more CAL. **(*Armitage 2004*)**

Aggressive periodontitis is much less common than chronic periodontitis representing only one to two percent of all cases of periodontitis. Aggressive periodontitis occurs in either localized or

generalized forms with both conditions affect usually apparently healthy patients, although abnormalities in phagocyte function can be found. Elevated proportions of *Aggregatibacter Actinomycetemcomitans* (AA) and in some populations *Porphyromonas Gingivalis* (PG) may be found. Overstimulated macrophages were reported to excrete elevated levels of Prostaglandin E2 and Interleukin-1β in cases of aggressive periodontitis. Rapid loss of attachment and rapid bone destruction occurs in a short period of time, but the condition may be self-arresting. Severity of disease is not related to the amount of bacterial plaque present and there is a strong genetic predisposition. **(*Yang et al., 2005*)**

Localized aggressive periodontitis (LAP) usually occurs circumpubertal with localized first molar-incisor presentation with interproximal attachment loss on at least two permanent teeth one of which is the first molar and involving not more than two teeth other than first molars and incisors. The bone loss may be rapid and severe with few obvious gingival inflammation and minimal amount of plaque. **(*Lang et al., 1999*)**

Generalized aggressive periodontitis (GAP) may affect adolescents and younger adults with age ranges between 25 to 35 years. Generalized aggressive periodontitis affects the entire dentition including teeth other than molars or incisors. Large

accumulations of plaque and calculus may be present. Inflammation may be severe and bone loss is rapid with a pronounced episodic nature of the destruction. **(*Lang et al., 1999*)**

The chronic persistence of subgingival plaque bacteria can lead to inflammation and tissue damage which results in cytokines release in periodontal tissues. Cytokines has a leading role at all stages of the immune response in periodontal disease. Components of the cell wall of pathogenic bacteria stimulate the production of proinflammatory cytokines release from the host immune cells (via toll-like receptor-2 and toll-like receptor-4), these cytokines include interleukin-1beta (IL-1β) and tumor necrosis factor-alpha (TNF-α) resulting in alveolar bone resorption and the production of matrix metalloproteinases (MMPs) that are responsible for degradation and loss of connective tissue of the periodontal ligament fibers. (***Ford et al., 2010)***

Cytokines are soluble proteins which are secreted by cells to act as a messenger that transmits signals to other cells. They initiate, mediate and control immune and inflammatory responses. They also regulate growth and differentiation of cells. Macrophages produce a broad range of cytokines which are interleukin- 1 alpha (IL-1 α), interleukin-1beta (IL-1β), tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6) and interferon gamma (IFN-γ) which are the pro-inflammatory cytokines, whereas macrophages produce also

interleukin-4 (IL-4) and interleukin-10 (IL-10) which are the anti-inflammatory cytokines. Both types of cytokines often found to balance each other as they stimulate cell production and affect communication between cells. **(*Elankov et al., 2005*)**

Cytokines functions include leukocytes direction to respond to microbial stimuli, upregulate expression of adhesion molecules on migrating leukocytes, activation of kinins, arachidonic acid derivative, prostaglandins and leukotrienes and increase the release of reactive oxygen intermediates, nitric oxide, vasoactive amines and neuropeptide. Also, cytokines regulate the expression of complement processing and scavenger receptors: NOD like receptor and Toll like receptors. Cells involved in innate immune response include neutrophils, natural killer cells, macrophages, mast cells and eosinophils produce and respond to cytokines generated within seconds of tissue insult. **(*Gemmell and Seymour 2004*)**

The most commonly blamed cytokines for periodontal destruction are interleukin-1beta (IL-1β), tumor necrosis factor alpha (TNF-α), and prostaglandin E2. The interleukin-1 family consists of three homologous proteins: IL-1α and -1ß which are pro-inflammatory proteins, and IL-1 receptor antagonist (IL-1RN) which is an antagonist protein to IL-1. IL-1ß is generally found in the circulation or tissue fluids and IL-1α is retained by the cell in intracellular and membrane bound forms. The cytokines IL- 1α, IL-

1β and IL1-RN are encoded by the genes IL-1A, IL-1B and IL-1RN which are located in close proximity in the IL-1 gene cluster on chromosome 2q13–21. ***(Nares 2003)***

The interleukin-1 family has an important key role in innate and adaptive immunity and in pathogenesis of infectious, autoimmune and inflammatory diseases. Clinically elevated levels of IL-1 mostly IL-1ß have been associated with many diseases and higher gingival fluid levels of IL-1 have been associated with periodontitis severity. Trials of blocking IL-1 activity are done as a therapy for some inflammatory diseases like rheumatoid arthritis. ***(Nares 2003).***

Interleukin-1β is a proinflammatory cytokine secreted from stimulated monocytes, macrophages, keratinocytes, smooth muscles & endothelial cells. It is present in high levels in the gingival crevicular fluid & gingival tissues of periodontitis cases. Interleukin-1β is associated with destruction of extracellular matrix & bone resorption in periodontal diseases. It represents a primary activator of early chemotactic cytokines, as well as of the expression of adhesion molecules that allow migration of leucocytes into tissues. It also stimulates osteoclastic activity resulting in bone resorption. IL-1β triggers different enzymes leading to the production of prostaglandin E2 and is a primary regulator of matrix metalloproteinases and their inhibitors. Among the pro