Factors Modulating Responses of Oral Hygiene Instructions

by

Avinash Akkur

Master of Dental Surgery, GITAM Dental College and Hospital, Dr. NTR University of Health Sciences, Andhra-Pradesh, India 2016

Submitted to the Graduate Faculty of the

School of Dental Medicine

Master of Science

University of Pittsburgh

2023

UNIVERSITY OF PITTSBURGH

SCHOOL OF DENTAL MEDICINE

This thesis was presented

by

Avinash Akkur

It was defended on

April 6, 2023

and approved by

Alexandre R Vieira, DDS MS PhD, Oral and Craniofacial Sciences

Mariana Bezamat Chappel, DDS PhD, Oral and Craniofacial Sciences

Konstantinos Verdelis, DDS PhD, Oral and Craniofacial Sciences

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Avinash Akkur, B.D.S, M.D.S, M.S

University of Pittsburgh, 2023

Background: High Blood Pressure (HBP) and Periodontal disease (PD) are prevalent chronic disorders. HBP is known to impact cardiovascular disease (CVD) complications significantly, and PD has also been vastly studied regarding its potential involvement with CVD. The present study analyses the association of risk factors like hypertension and pro-inflammatory cytokines like IL-1a and IL-1b in the subjects who have received non-surgical therapy and oral hygiene instructions for periodontitis.

Methods: Clinical data and genomic DNA samples (saliva) were obtained from the University of Pittsburgh Dental Registry and DNA Repository (DRDR) project. A total of 578 subjects from both sexes were considered by checking their periodontal and hypertension status using an electronic health record system. All the participants were divided into four groups. Group A: consists of healthy subjects without PD and HBP; Group B: subjects with PD and HBP; Group C: subjects with PD but without HBP; Group D: subjects without PD but having HBP. They were examined on more than one visit to assess their periodontal status after non-surgical intervention and oral hygiene instructions. The patients were classified as Improved, Worsened, and Neutral based on their plaque score before and after the intervention. We genotyped all the samples using SNPs rs1800587 (*IL-1a*) and rs1143634 (*IL-1b*). We studied the association of *IL-1* with periodontitis and hypertension by comparing groups B, C, and D with group A, and its sex predilection was also analysed. In addition, we studied the role of *IL-1a* and *IL-1b* in affecting the oral hygiene status of the subjects by comparing their plaque indices from the first and second visits.

Results: *IL-1a* was associated with subjects having only PD ($p<0.05$), and *IL-1b* was associated with subjects having both PD and HBP ($p<0.05$). The oral hygiene status showed a significant improvement after non-surgical therapy and oral hygiene instructions in subjects with both PD and HBP and was associated with the *IL-1b* ($p<0.05$). However, there was no sex predilection ($p >0.05$).

Conclusion: Our data showed that despite the multifactorial nature of PD, a significant improvement could be noticed in subjects undergoing non-surgical intervention and strictly following oral hygiene instructions.

Table of Contents

List of Tables

List of Figures

Preface

I would like to thank all those who made my Master of Science degree possible. First and foremost, I am extremely thankful to the Almighty for showering his infinite blessings on me. Next, I am very grateful to my advisor and mentor Dr. Alexandre Vieira. Through his guidance, I have been able to achieve greater heights in my life. Thank you very much Dr. Vieira for all the support and confidence that you have rendered on me during my tough times. It means a lot to me.

I would also thank my committee members, Dr. Mariana Bezamat and Dr. Konstantinos Verdelis for being a part of my project and helping me in understanding intricate details about the topic, and for making this project a successful one.

I also gratefully acknowledge other members of the Vieira lab: Kathleen Deeley, Teegan, Rebecca who assisted and guided me with my bench work. Additionally, I would like to thank Elaine Dizak for being always there for me.

I am blessed to have my parents A. Krishna Mohan, A. Anuradha for always standing by me and supporting me during every phase of my life. I am thankful to my brothers Abhishek and Karthik, my aunt Leelavati for helping me when needed. My sincere thanks to My Wife K.Aparna for joining me at Pittsburgh from July 2022, and being a part of my hardships. Last, but not the least all this wouldn't have been possible without my loving daughter Advaita Nayana. Her presence around me inspired me to work hard each day. Thank you, GOD, for blessing us with her. She made our life complete.

1.0 Introduction

1.1 Periodontitis and Its Classification

Periodontal Disease (PD) or Periodontitis is a destructive disease that affects the attachment apparatus of the tooth namely the periodontal ligament (PDL), cementum and the alveolar bone (Macedo Paizan & Vilela-Martin, 2014). It is one of the most common inflammatory conditions worldwide, and it is regarded as the 6th most common inflammatory condition with a prevalence of 20-50%. Periodontitis is characterized by microbially, and host mediated inflammation that results in loss of periodontal attachment. The chief pathophysiological aspect of this disease is the activation of host-derived proteinases that causes loss of the bone and apical migration of the junctional epithelium. According to the 1999 classification system that focused on the unique features of different periodontitis phenotypes. This led to the recognition of four different forms of periodontitis (Tonetti, Greenwell, & Kornman, 2018):

1. Necrotizing periodontitis
2. Chronic periodontitis
3. Aggressive periodontitis
4. Periodontitis as a manifestation of systemic diseases.

This classification has distinguished between aggressive periodontitis and chronic periodontitis. Chronic periodontitis affects high proportion and is characterized by slow progression. However, Aggressive periodontitis is defined as a rare inflammatory condition characterized by rapid and severe destruction of the connective tissue attachment and bone with

minimal presence of the microbial deposits that affected younger individuals with familial aggregation (Brodzikowska, Górski, & Bogusławska-Kapała, 2022).

Major changes were made since the 1999 workshop, substantial new information has emerged from the population studies, basic science investigations, and from the environmental and systemic risk factors. This analysis has prompted for the 2017 workshop to develop a new classification framework for periodontitis (Caton et al., 2018). The staging is largely dependent upon the severity of the disease during initial presentation and well as the complexity of the disease management. While the grading provides supplemental information about biological features of the disease progression, assessing the risk of further progression, and anticipated poor outcomes of the treatment (Tonetti et al., 2018). Staging involves four categories (stage 1 through 4) and it is determined by considering several variables including clinical attachment loss, amount of bone destruction, probing depth and the presence of periodontal pockets.

a. Stages – Based on severity and complexity of management (Caton et al., 2018)
   Stage 1- Initial Periodontitis

   Stage 2- Moderate Periodontitis

   Stage 3- Severe Periodontitis with potential for additional tooth loss

   Stage 4- Severe Periodontitis with potential for loss of the dentition

The grading includes three levels (grade A through grade C) which encompasses, in addition to aspects related to periodontitis progression, general health status, and other exposures such as smoking or level of metabolic control. Therefore, grading allows the clinician to incorporate individual patient factors into diagnosis, which are crucial to comprehensive case management.

b. Grades- Evidence or risk of rapid progression, anticipated treatment response (Caton et al., 2018)

Grade A- Slow rate of progression

Grade B- Moderate rate of progression

Grade C- Rapid rate of progression

1.2 Hypertension

Hypertension is defined as the systolic BP > 140 mm Hg and diastolic BP > 90 mm Hg (Del Pinto et al., 2020). Hypertension is one of the most common causes for premature death and disability. It is the major contributor of the cardiovascular disease including coronary heart diseases and stroke. It is important to evaluate the relationship between hypertension and periodontal disease (Kawabata et al., 2016). Hypertension is considered a condition of low-grade inflammation causing the activation of the adaptive immune system. Several studies have shown the involvement of T cells which gets activated and accumulate in the perivascular tissue (Del Pinto et al., 2020).

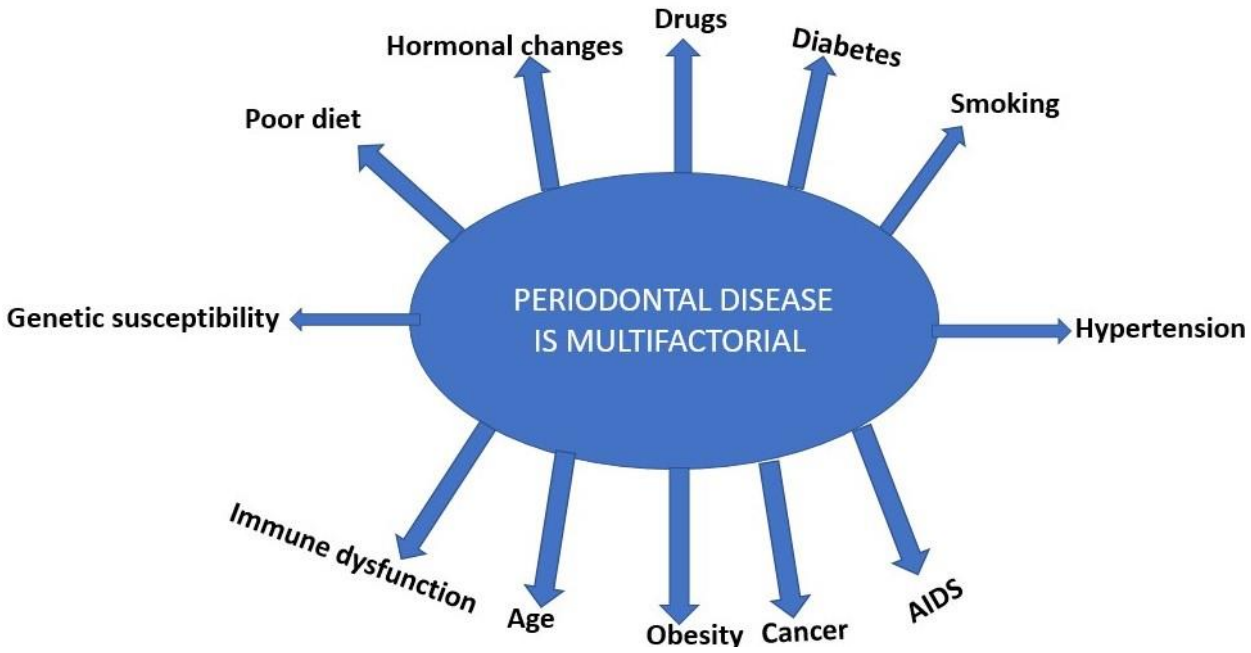

Figure 1: Etiological factors of periodontal disease (Macedo Paizan & Vilela-Martin, 2014).

1.3 Association Between Periodontitis and Hypertension

The inflammation caused during periodontal infections is not only restricted to the oral cavity, but it also affects the endothelium of the blood vessels (Czesnikiewicz-Guzik et al., 2019) (Muñoz Aguilera et al., 2020). Therefore, periodontitis may be a significant contributor to cardiovascular diseases, endothelial dysfunction, and both of which are potential causes of hypertension (Figure 1). High blood pressure promotes immune-cell activation, and it also increases inflammatory mediators to promote tissue entry of activated inflammatory cells. These activated immune cells triggers an inflammatory response that disrupts functions that regulate normal blood pressure. This leads to hypertension (Figure 2). Therefore, chronic inflammatory disorders like periodontitis may form a foundation for pro-hypertensive inflammation (Yildirim et al., 2022). There are several interventions to treat oral inflammation, and prevention of

hypertension and its complications. Few studies have stated that moderate-severe periodontitis is associated with increased odds for hypertension (Del Pinto et al., 2020).

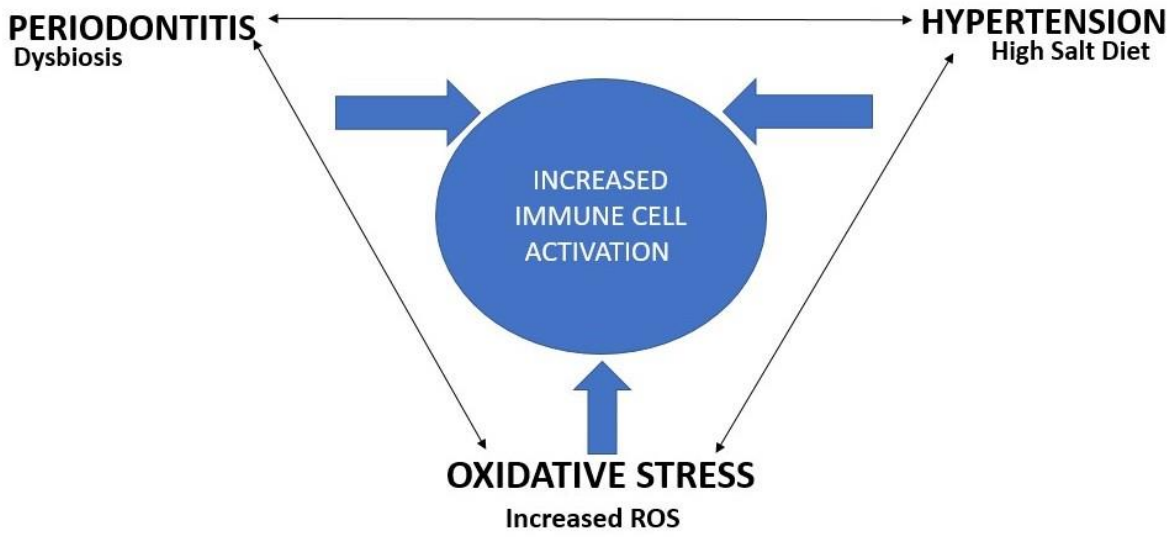

Figure 2: Interrelationship between periodontitis, hypertension, and oxidative stress (Del Pinto et al., 2020).

1.4 Interleukin -1

Interleukin -1 (IL-1) is a family of at least ten molecules, of which the two most significant ones in the pathogenesis of periodontitis are IL-1a which is connected to the cell, while the IL-1b which is released into the environment and showing agonistic action upon binding with the receptor (Brodzikowska, Górska, & Kowalski, 2019). They are special subgroups of cytokines that are associated with periodontal inflammation. Among the different kinds, Interleukin -1 (IL-1) is an important inflammatory mediator in periodontitis. Apart from IL-1a and IL-1b , there is also a third form which is the ligand IL-1 receptor antagonist (IL-1 ra) which functions as a competitive

inhibitor (Grigoriadou, Koutayas, Madianos, & Strub, 2010). The IL-1a and IL-1b are encoded respectively by the *IL-1a* and *b* genes located on the long arm of chromosome 2 and having common DNA sequences. The other cytokine namely the Tumor necrosis factor alpha (TNF alpha) along with Interleukin I (IL-1) are the key mediators of periodontal inflammation (Kornman et al., 1997).

1.4.1 INTERLEUKIN-1a (*IL-1a*) rs1800587, and INTERLEUKIN-1b (*IL-1b*) rs1143634

Interleukin-1a (*IL-1a*) rs1800587 together with Interleukin-1b (*IL-1b*) rs1143634 are considered as a predictive factor for severe course of chronic periodontitis. Several studies have shown that rs1800587 alone and together with rs1143634 are associated with the clinical parameters of periodontitis (Brodzikowska et al., 2019). The *IL-1a* is known to drive type 3 immunity through assisting IL-23 and IL-6 in the activation of Th17 cells and expression of IL-17. The *IL-1b* has shown to be induced upon host-microbiota interaction and causes the expansion and activation of both Th1 and Th2 cells (Pan, Wang, & Chen, 2019).

1.5 Association Between Interleukin-1 and Periodontitis

Periodontal diseases are predominantly caused by periodontal pathogens especially the ones in the red complex namely *Porphyromonas gingivalis, Tannerella forsythia,* and *Troponema denticola,* and the *Aggregatibacter actinomycetemcomitans*. These pathogens trigger the expression of proinflammatory cytokines such as Interleukin-1(IL-1) which is associated with immunopathology of periodontal infection. (Ferreira et al., 2008). Because differences in the

amount or function of IL-1 produced in response to a bacterial agent may potentially contribute to differences in susceptibility to periodontitis and the course of the disease. Therefore, the polymorphisms of the IL-1 encoding genes, as susceptibility markers for periodontitis has become an area of interest among the researchers(Brodzikowska et al., 2019). Gene polymorphisms encoding Interleukin -1 (IL-1) are the most prominent gene polymorphisms in studies on periodontitis(Kinane, 2001; Seneviratne, Zhang, & Samaranayake, 2011).

1.6 Classification of Soft Deposits on the Tooth (Schwartz & Massler, 1969)

1. Acquired pellicle – A non -cellular thin film.
2. Dental Plaque- An organized transparent deposit which is primarily composed of bacteria and their products.
3. Material Alba- Soft, whitish deposit with no specific architecture, which can be removed by water spray.
4. Food debris- Retained food which is usually removed by saliva and oral muscular action.

1.7 Dental Plaque and Its Composition

According to Carranza, 11 th edition (Newman, Takei, Carranza, & Klokkevold, 2012) dental plaque is defined clinically as a structured, resilient yellow-greyish substance that adheres tenaciously to the intraoral hard surfaces, including removable and fixed restorations.

Dental plaque consists of:

1. Solids 20-30 %
2. Water 70-80%

The solid portion of the dental plaque chiefly consists of:

1. Micro-organisms – 70 % both bacterial and non-bacterial organisms.
2. Intracellular Matrix- 20-30 % consisting of both organic and inorganic materials.

It is noteworthy that the composition and properties of the dental plaque could lead to diseases, such as dental caries and periodontal diseases. It is really important for a clinician to be aware about the advances in the field of dental plaque for better treatment options in the future (Seneviratne et al., 2011).

1.8 Classification of the Dental Plaque and Its Role in Pathogenesis of Periodontal Disease and Systemic Inflammation

Dental plaque can be classified as:

1. Supragingival plaque is found at or above the gingival margin. If the supragingival plaque is in direct contact with the gingival margin is referred to as marginal plaque.
2. Subgingival plaque is found below the gingival margin, between the tooth and gingival sulcular tissue.

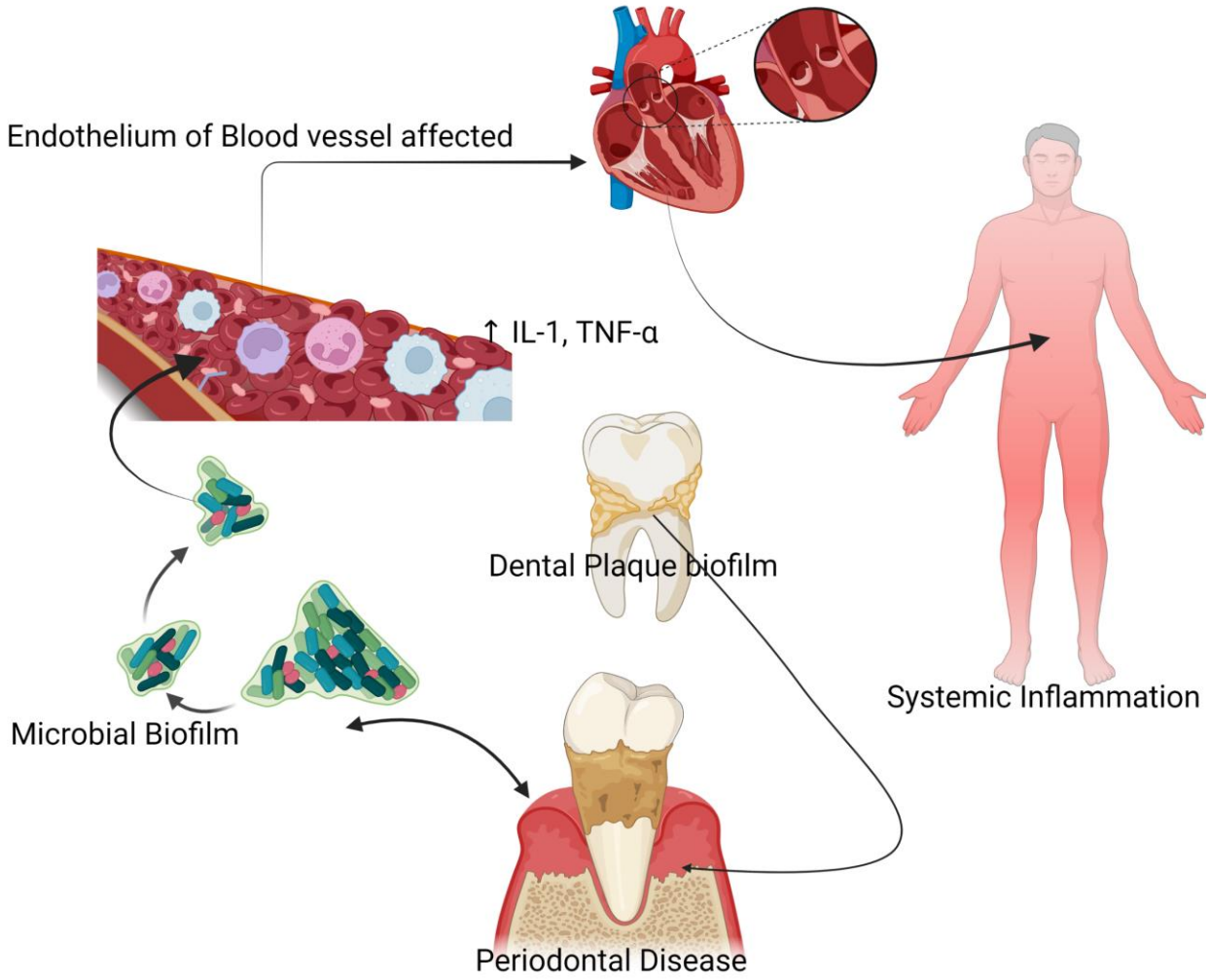

Figure 3: Role of dental plaque in the pathogenesis of periodontal disease and systemic inflammation (Havemose-Poulsen, Sørensen, Bendtzen, & Holmstrup, 2007).

Biofilm lodges in the crevices around the teeth both above and below the gingival margin. Accumulation of dental plaque biofilm can result in dental caries and periodontal disease. Moreover, subgingival plaque bacteria and their products may gain access to distant sites in the body through the circulatory system any potentially contribute to systemic inflammation (Figure3). This happens in the following steps:

1. The interleukin-1 beta, prostaglandins, tumor necrosis factor alpha (TNF-alpha), and matrix metalloproteinases are mediators that recruit neutrophils to the area via chemotaxis and cause increased permeability of gingival blood vessels.
2. As the gingival inflammatory process continues, additional mediators are produced, and more inflammatory cells such as neutrophils, T cells, and monocytes are recruited to the area.
3. Proinflammatory cytokines are produced in the tissues as a response to the chronic inflammatory process, and these proteins may further escalate the local inflammatory response and affect the initiation and progression of systemic inflammation and disease.
4. The result of this chronic inflammation is a breakdown of gingival collagen and accumulation of an inflammatory infiltrate leading to the clinical signs of gingivitis.
5. In some individuals, the inflammatory process will also lead to the breakdown of collagen in the periodontal ligament and resorption of the supporting alveolar bone. It is at this specific point; the lesion progresses from gingivitis to periodontitis.
6. Therefore, controlling dental plaque biofilm is essential to preventing and reversing gingivitis as well as preventing and managing periodontitis.

1.9 Formation and the Structure of Dental Plaque Biofilm

The oral cavity is exposed to different microbiota like other regions of the body. These microbiotas are distributed in the stratified squamous oral mucosal surfaces, tooth surfaces and other muco-gingival margins. The dental plaque is an archetypical biofilm formed by association between complex group of microorganism (Seneviratne et al., 2011).

It is well known that the adherence of microorganism to the oral surfaces is an important prerequisite for the formation of the dental plaque. These microorganisms form discrete microcolonies. These microcolonies secrete extracellular polymeric substance (EPS), which is a

distinctive feature seen in microbial biofilm and provides a physical scaffold for the biofilm community. It also has some special properties like the presence of antimicrobial enzymes which protects the microorganisms from noxious external stimuli. In course of time, the microorganisms which have adhered to the EPS will form a three-dimensional spatially arranged mature biofilm community.

The sequence of events in the formation of the dental plaque biofilm (Figure 4) (Seneviratne et al., 2011):

1. Bacteria and some of the components in saliva adhere to the cleaned tooth surface.
2. These products tend to be absorbed by the negatively charged hydroxyapatite crystals of the enamel making a thin layer of conditioning film called the 'acquired pellicle.'
3. The acquired pellicle in a supra-gingival plaque tends to be covered by positively charged molecules such as salivary glycoproteins, statherian, histatin, proline-rich proteins, alpha-amylase. It also has some products from the gingival crevicular fluids.
4. The acquired pellicle consisted of the gram-positive bacteria like the *Streptococcus sanguinis*, *Streptococcus oralis*, *Streptococcus mitis*, and *Neisseria* species as primary colonizers.
5. After the formation of the first layer of primary colonizers, the dental plaque biofilm continues to build up by multiplication of the primary colonizers, and secondary colonizers continue to co-aggregate and lead to co-adhesion.
6. The secondary colonizers can attach to the receptors of the primary colonizers.
7. The primary colonizers of the dental plaque are either aerobic or facultative aerobes, such as the *Streptococcus* and *Fusobacterium* groups of bacteria. They reduce the oxygen, which favors the anerobic bacteria to enter the biofilm community as secondary colonizers (Marsh, 2005).
8. The secondary colonizers are mainly gram-negative species such as *Actinomyces* species, *Fusobacterium nucleatum*, *Prevotella intermedia*, and *Capnocytophaga* species.

9. Coaggregation occurs between Gram-positive species such as *Streptococcus sanguinis*, and *Actinomyces*; between Gram-negative species, such as *Streptococcus*; and *Fusobacterium* respectively.
10. Some specific structural features of the dental plaque such as the ‘corn-cob’ and ‘test-tube brush’ appearance can be observed due to the adherence of the cocci to filamentous bacteria.

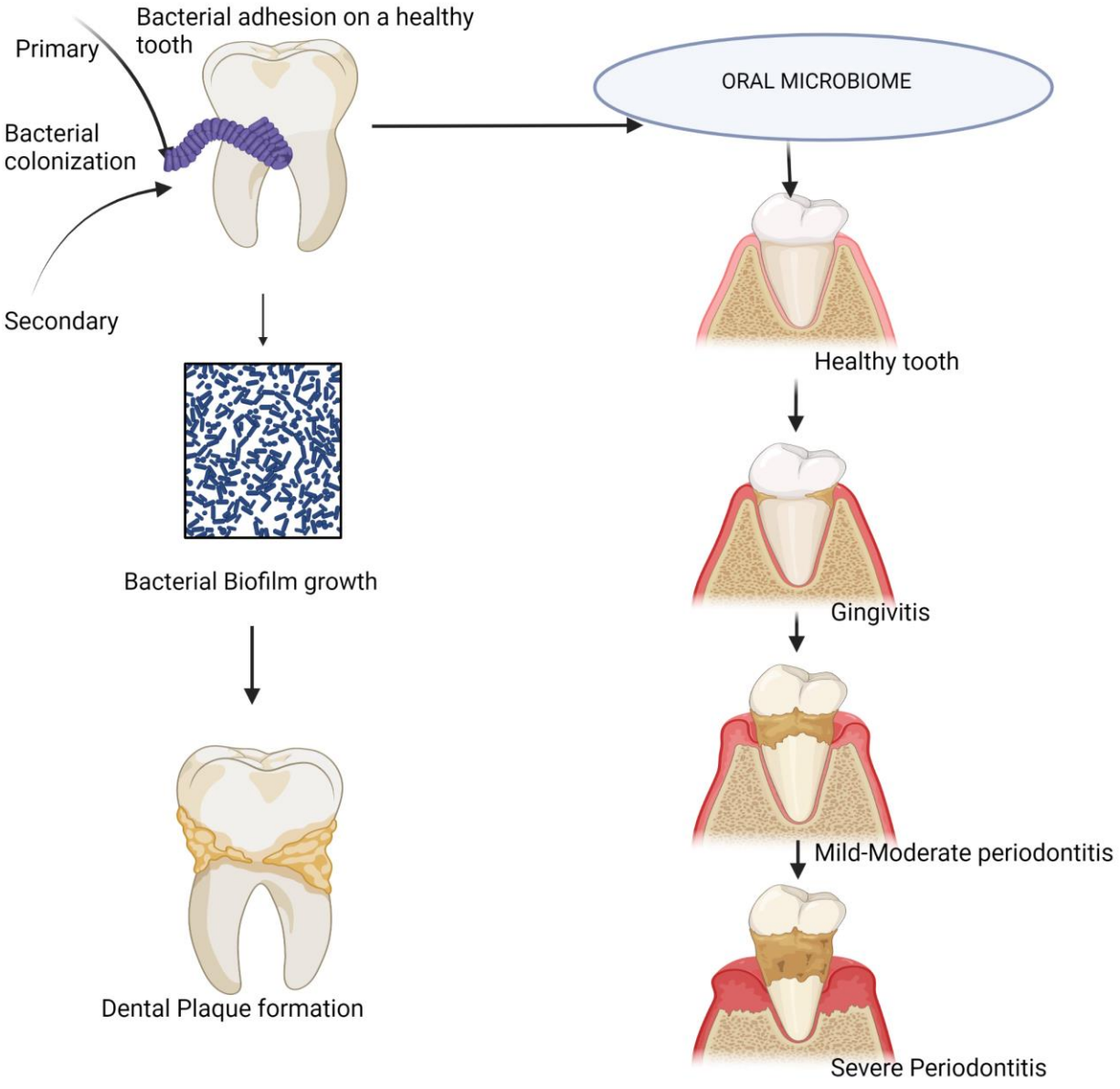

Figure 4: Sequence of events in the formation of mature biofilm and dental plaque (Seneviratne et al., 2011).

1.10 Fate of the Dental Plaque

If the dental plaque biofilm is left undisturbed for approximately 7 days, the local environment rapidly changes and it favour's the colonization by some gram-negative anaerobic

species known as ‘tertiary colonisers’ (Marsh, 2005). The tertiary colonisers consists primarily pathogenic bacteria such as *Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans,* and spirochetes such as *Troponema denticola* (Seneviratne et al., 2011). The dental plaque biofilm matures by 72 hours although this timing could be altered by factors such as food habits and the immune response of the host.

1.11 Quorum Sensing (QS)

Quorum sensing (QS) is also known as communication between bacteria is an important property seen within the bacteria in the bacterial biofilm. This property helps in controlling the growth of the microbial community by signalling to bacteria to leave the biofilm to enter a new habitat. This is chiefly mediated by small molecules such as competence stimulating peptide (CSP) and autoinducer-2 (AI-2) which are primarily involved in communication between different species of bacteria. This leads to virulence traits by gene transfer, particularly the antibiotic-resistance genes (Seneviratne et al., 2011).

1.12 Silness-Loe Plaque Index

Table 1: Silness & Loe Plaque Index used as a criterion for determining dental plaque of the subjects (Loe & Silness, 1963).

0	Absence of microbial plaque
1	Thin film of microbial plaque along the free gingival margin
2	Moderate accumulation with plaque in the sulcus
3	Large amount of plaque in sulcus or pocket along the free gingival margin

1.13 Oral Hygiene Instructions

- Dental plaque control is one of the most important factors in preventing periodontal disease (PD).
- Poor oral hygiene and accumulation of subgingival biofilm results in inflammation in the gingival and periodontal tissue.
- Initial therapy of a periodontal disease (PD) is not only targeting non-surgical interventions like scaling and root planning, but also on behavior change and motivation of the patient to maintain proper oral hygiene. This is achieved in response to ***oral hygiene instructions.***
- In our study, oral hygiene instructions were provided to the patients, the same time at the initial appointment.
- Students are trained to present all the required oral hygiene instructions to the patients after non-surgical intervention.
- Recording plaque indices before and after oral hygiene instructions offers an opportunity to motivate the individual, pointing to specific sextants that scored worse, thereby preventing future periodontal breakdown.

2.0 Aims

The aims of this master's thesis were to:

2.1 AIM1: Define Cohorts Depending on Periodontal and Hypertension Status From the Dental Registry and DNA Repository (DRDR) Project.

Aim 1 established the sample that was chosen from the University of Pittsburgh School of Dental Medicine from the Dental Registry and DNA Repository Project. The sample size was 578 subjects 25-85 years of age.

Of this, the subjects who had periodontitis were diagnosed having moderate to severe periodontitis after more than one periodontal evaluation, and the subjects having hypertension were diagnosed as hypertensive based on their medical and drug history. The controls included in the study did not have either PD or HBP, and subjects having PD and HBP were randomly selected from DRDR with a total of 7000 individuals and separated them into three groups.

2.2 Aim2: The Frequency of *IL*-1a and *IL-1b* Genotypes Considering Sex, Periodontal and Their Hypertension Status.

- Aim 2 uses the periodontal and hypertension status of male and female subjects and determines the frequency of alleles of the Interleukin-1 (*IL-1a and IL-1b*) gene in these subjects. The subjects were identified, and their saliva samples was used to extract genomic

DNA and determine genotypes to test for *IL-1a* and *IL-b* association in these subjects using SNPs **rs1800587 (*IL-1a*) and rs1143634 (*IL-1b*).**

- DNA of the subjects were aliquoted in the Microamp Optical 96-Well Reaction Plate. Samples were transferred from the 96-well dilution plates to the 384- well reaction plates.
- The PCR reaction was performed with 1.5 µl of TaqMan Universal Master Mix and 0.462 µl of Sterile water and 0.037 µl of both the SNPs separately (rs1800587 and rs1143634).
- Negative control (water) was included in all plates.
- The DNA samples were amplified using Gene Amp* PCR System 9700.
- <u>The following program was used:</u>
- 95℃ for 10 minutes, 40 cycles of (92 ℃ for 15 seconds and 60 ℃ for 1 minute).
- Genotyping experiments were performed to detect single nucleotide polymorphism (SNP) variants of a target nucleic acid sequence in the samples.
- In this study, genotyping was done using the TaqMan SNP genotyping assay which detects variants of a single nucleic acid sequence and end-point analysis.
- The presence of two probes (*VIC and FAM*) in each assay allows genotyping of the two possible variants at the SNP site in a target sequence.
- Genotyping of the amplified DNA was done on *Quant Studio 6 Flex machine*. This machine genotypes all the DNA samples from the reaction plate simultaneously.
- First, the software normalizes the fluorescence of the reporter dyes (*VIC* and *FAM*) to the fluorescence of the passive reference dye in each well.

- Next, the software plots the normalized intensities of the reporter dyes in each sample well on an allelic discrimination plot which contrasts the reporter dye intensities of the allele specific probes.

In this research experiment, we hypothesize that:

1. *IL-1a* is associated with subjects with only periodontitis, and it is not associated with hypertension or in the subjects with both periodontitis and hypertension.
2. *IL-1b* is associated with subjects with having a combination of both periodontitis and hypertension. It is not associated in subjects with only periodontitis or in the subjects with only hypertension. Hence, *IL-1b* is associated in causing periodontitis in patients with hypertension or vice-versa.
3. There is no associated sex predominance of *IL-1a* or *IL-1b*.
4. The homozygous dominant gene (GG) was the most common allele among both males and females in all the four groups after genotyping with both the SNPs.

2.3 AIM3: Test if Different Oral Hygiene Responses are Associated with Interleukin-1 Genotypes.

Aim 3 classifies subjects as Improved, Worsened, and Neutral by comparing their plaque index before and after oral hygiene response. The association of Interleukin-1(*IL-1 and IL-1b*) gene in these subjects were determined.

In this research experiment, we hypothesize that:

1. When the subjects from Group A was compared with Group B, C, and D there was a significant improvement in the oral hygiene status of all the subjects.
2. IL-1b genotypes of the subjects from Group B and Group C have shown a significant improvement of oral hygiene status following non-surgical therapy.

3.0 Results

3.1 Classification of the Subjects into Cohorts

Group A: healthy subjects without PD and HBP.

Group B: subjects with PD and HBP.

Group C: subjects with PD, but without HBP.

Group D: subjects without PD but having HBP.

Table 2: Total number of male & female subjects in all the groups

GROUP	MALE	FEMALE	TOTAL
A	33	37	70
B	93	76	169
C	136	117	253
D	34	52	86

3.2 Genotypes Configuration

GA-Heterozygous

GG-Homozygous dominant

AA-Homozygous recessive

3.3 Interleukin-1 Markers

rs1800587 - *IL-1a*

rs1143634 - *IL-1b*

Table 3: Total Number of Subjects with the following Combination of Genotypes from Group A When Genotyped with Both the SNPs.

ALLELES *OF IL-1a +IL-1b*	MALES	FEMALES	TOTAL	%
GGGG	12	9	21	36.8
GGGA	2	0	2	3.5
GGAA	1	0	1	1.7
GAGG	5	6	11	19.2
GAGA	3	6	9	15.7
GAAA	2	2	4	7.0
AAGG	0	0	0	0
AAGA	2	3	5	8.7
AAAA	1	3	4	7.0

Table 4: Total Number of Subjects with the following Combination of Genotypes from Group B When Genotyped with Both the SNPs.

ALLELES OF *IL-1a +IL-1b*	MALES	FEMALES	TOTAL	%
GGGG	34	27	61	22.7
GGGA	4	5	9	3.3
GGAA	2	0	2	0.7
GAGG	14	17	31	11.5
GAGA	26	11	37	13.8
GAAA	10	1	11	4.1
AAGG	3	4	7	2.6
AAGA	4	3	7	2.6
AAAA	4	0	4	1.4

Table 5: Total Number of Subjects with the following Combination of Genotypes from Group C When Genotyped with Both the SNPs.

ALLELES OF *IL-1a +IL-1b*	MALES	FEMALES	TOTAL	%
GGGG	50	45	95	42.9
GGGA	4	2	6	2.7
GGAA	1	0	1	0.4
GAGG	14	21	35	15.8
GAGA	31	27	58	26.2
GAAA	4	2	6	2.7
AAGG	4	2	6	2.7
AAGA	4	2	6	2.7
AAAA	6	2	8	3.6

Table 6: Total Number of Subjects with The Following Combination of Genotypes from Group D When Genotyped with Both the SNPs.

ALLELES OF *IL-1a +IL-1b*	MALES	FEMALES	TOTAL	%
GGGG	15	17	32	36.7
GGGA	4	2	6	6.8
GGAA	0	1	1	1.1
GAGG	4	9	13	14.9
GAGA	18	10	28	32.1
GAAA	0	0	0	0
AAGG	0	2	2	4.5
AAGA	1	4	5	5.7
AAAA	0	0	0	0

Table 7: Genotype of The Subjects from Group A To Group D After Genotyping Them With rs1800587 (*IL-1a*).

GROUP	GG	AA	GA	TOTAL
A	29	17	24	70
B	70	25	74	169
C	115	28	110	253
D	39	13	34	86

Table 8: Genotype of The Subjects from Group A To Group D After Genotyping Them with rs1143634 (*IL-1 b*).

GROUP	GG	AA	GA	TOTAL
A	39	12	19	70
B	56	10	103	169
C	157	19	77	253
D	50	5	31	86

Table 9: Genotype of Male & Female Subjects from Group A When Genotyped With rs1800587 (*IL-1a*).

SEX	GG	AA	GA
MALE	17	3	13
FEMALE	15	6	16

Table 10: Genotype of Male & Female Subjects from Group A When Genotyped With rs1143634 (*IL-1b*).

SEX	GG	AA	GA
MALE	20	5	8
FEMALE	19	6	12

Table 11: Genotype of Male & Female Subjects from Group B When Genotyped with the SNP rs1800587 (*IL-1a*).

SEX	GG	AA	GA
MALE	41	11	41
FEMALE	33	13	30

Table 12: Genotypes of Male & Female Subjects from Group B When Genotyped with the SNP rs1143634 (*IL-1b*).

SEX	GG	AA	GA
MALE	53	7	33
FEMALE	52	3	21

Table 13: Genotypes of Male & Female Subjects from Group C When Genotyped with the SNP rs1800587 (*IL-1a*).

SEX	GG	AA	GA
MALE	77	15	44
FEMALE	51	16	50

Table 14: Genotypes of Male & Female Subjects from Group C When Genotyped with The SNP rs1143634 (*IL- 1b*).

SEX	GG	AA	GA
MALE	82	12	42
FEMALE	75	7	35

Table 15: Genotypes of Male & Female Subjects from Group D When Genotyped with The SNP rs1800587 (*IL-1a*).

SEX	GG	AA	GA
MALE	19	1	14
FEMALE	26	6	20

Table 16: Genotypes of Male & Female Subjects from Group D When Genotyped with the SNP rs1143634 (*IL-1b*).

SEX	GG	AA	GA
MALE	23	4	7
FEMALE	30	5	17

Table 17: Oral Hygiene Status of The Subjects Following Non-Surgical Techniques and Oral Hygiene Instructions.

GROUP	IMPROVED	WORSENED	NEUTRAL
A	30	28	12
B	63	100	6
C	99	151	3
D	29	51	6

Table 18: Genotype of the Subjects who Worsened following Non-Surgical Techniques and Oral Hygiene Instructions When Genotyped with SNP rs1800587 (*IL-1a*).

GROUP	GG	GA	AA
A	7	12	3
B	22	21	5
C	36	29	10
D	18	10	4

Table 19: Genotype of the subjects who Improved following Non-Surgical Techniques and Oral Hygiene Instructions When Genotyped with the SNP rs1800587 (*IL-1a*).

GROUP	GG	GA	AA
A	17	12	6
B	46	48	13
C	63	58	9
D	13	14	2

Table 20: Genotype of The Subjects Who Did Not Show Any Difference in Their Oral Hygiene Status (Neutral) Following Non-Surgical Techniques and Oral Hygiene Instructions When Genotyped with the SNP rs1800587 (*IL-1a*).

GROUP	GG	GA	AA
A	0	0	0
B	1	1	4
C	1	0	1
D	1	8	6

Table 21: Genotype of the Subjects who Worsened following Non-Surgical Techniques and Oral Hygiene Instructions When Genotyped with the SNP rs1143634 (*IL-1b*).

GROUP	GG	GA	AA
A	11	10	1

B	29	17	2
C	52	29	6
D	0	23	9

Table 22: Genotype of the Improved Subjects who Improved following Non-Surgical Techniques and Oral Hygiene Instructions When Genotyped with the SNP rs1143634 (*IL-1b*).

GROUP	GG	GA	AA
A	21	6	8
B	69	33	4
C	83	41	8
D	16	10	2

Table 23: Genotype of the Subjects Who Did Not Show Any Difference in Their Oral Hygiene Status (Neutral) Following Non-Surgical Techniques and Oral Hygiene Instructions When Genotyped with the SNP rs1143634 (*IL-1b*).

GROUP	GG	GA	AA
A	0	0	0
B	2	4	0
C	1	0	1
D	7	8	0

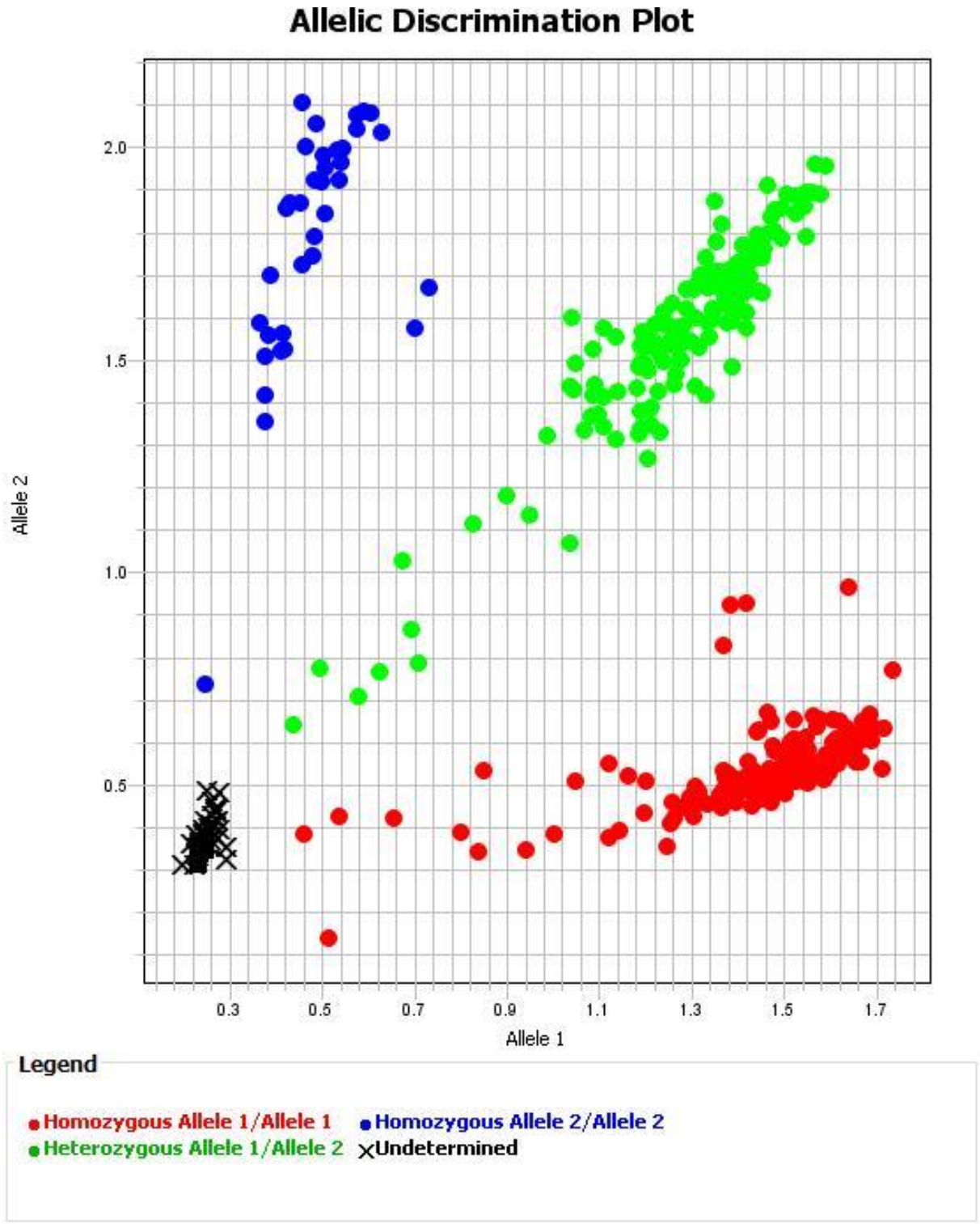

Figure 5: Plate 1-4 with SNP rs1800587.

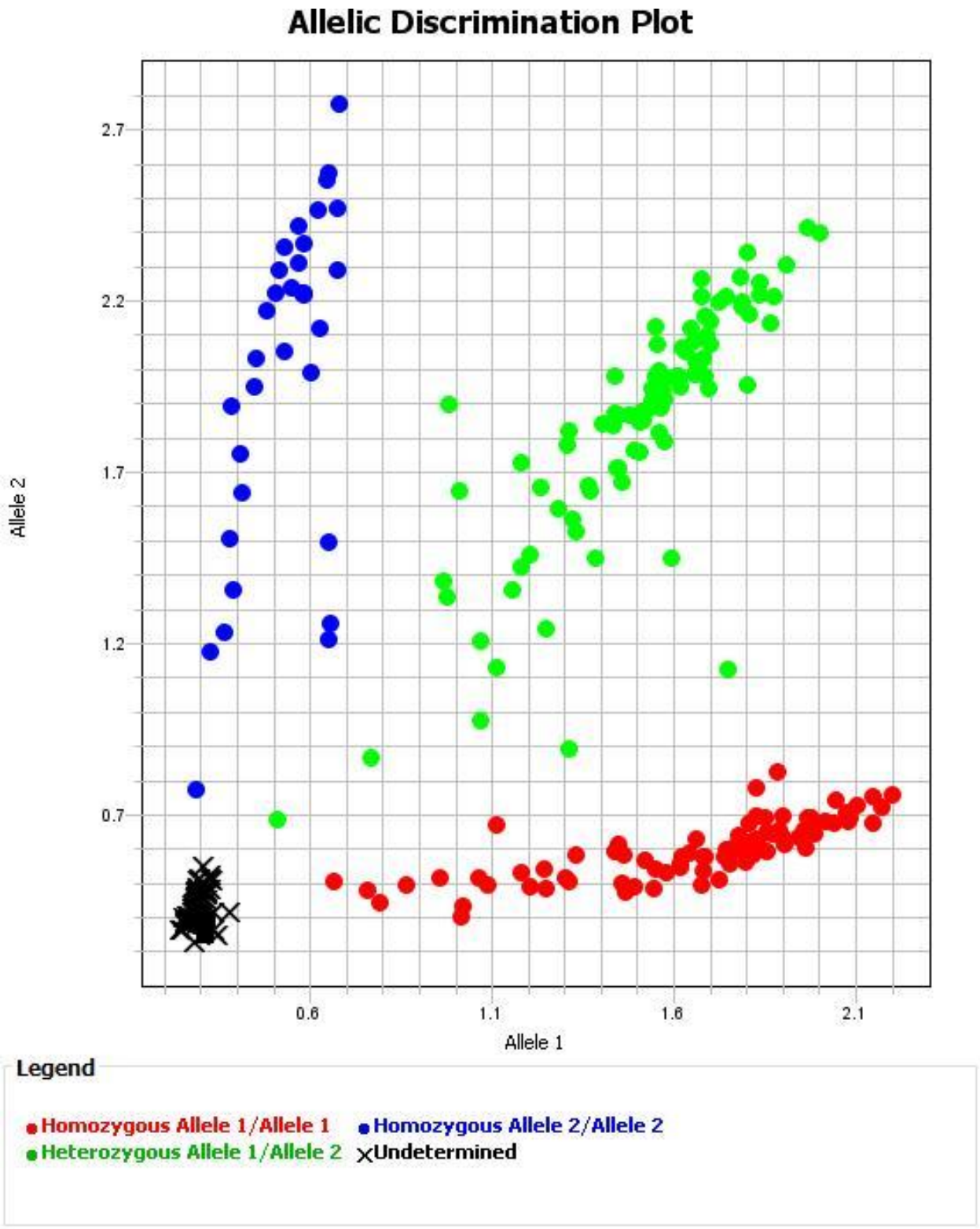

Figure 6: Plate 5-8 with SNP rs1800587.

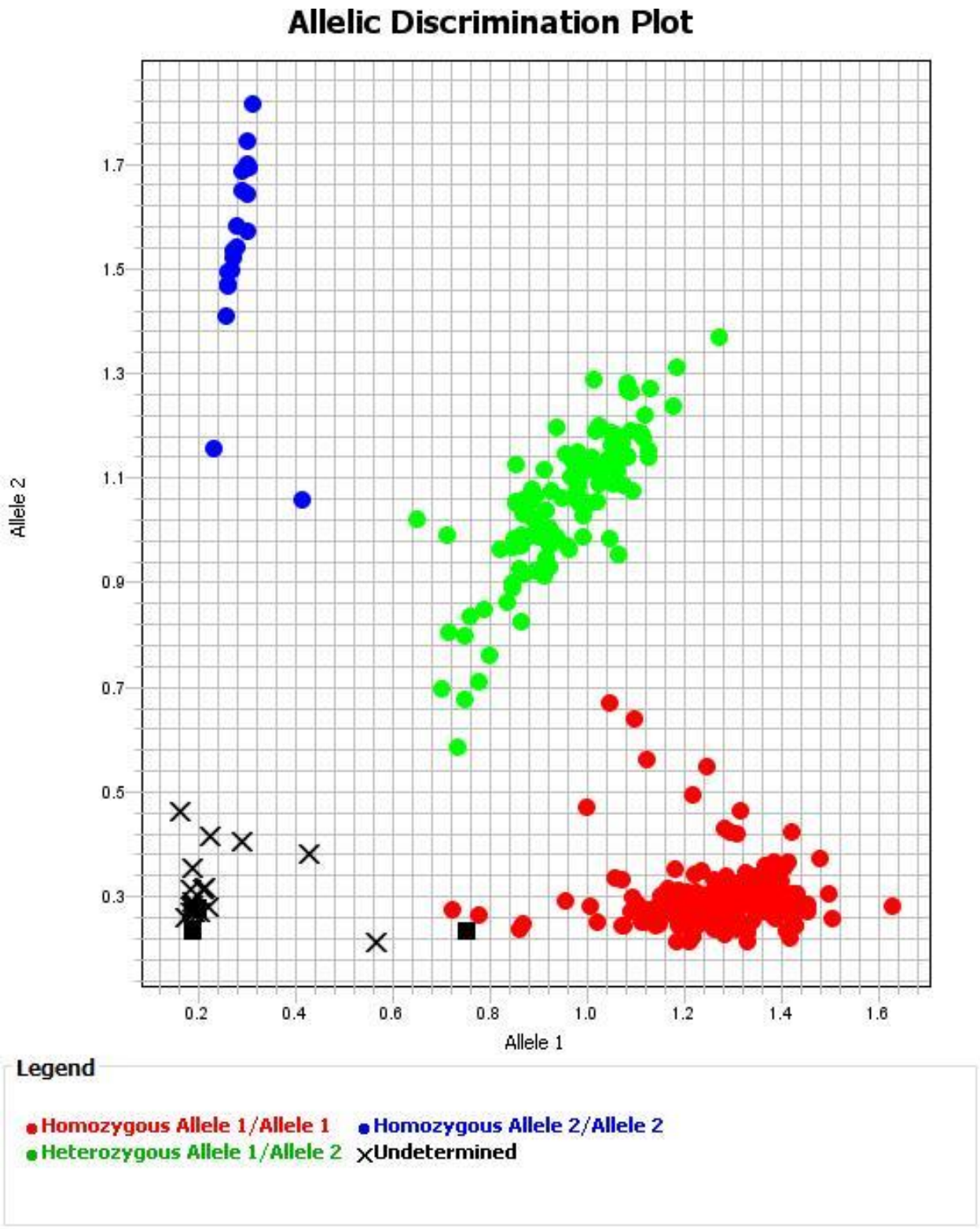

Figure 7: Plate 1-4 with SNP rs1143634.

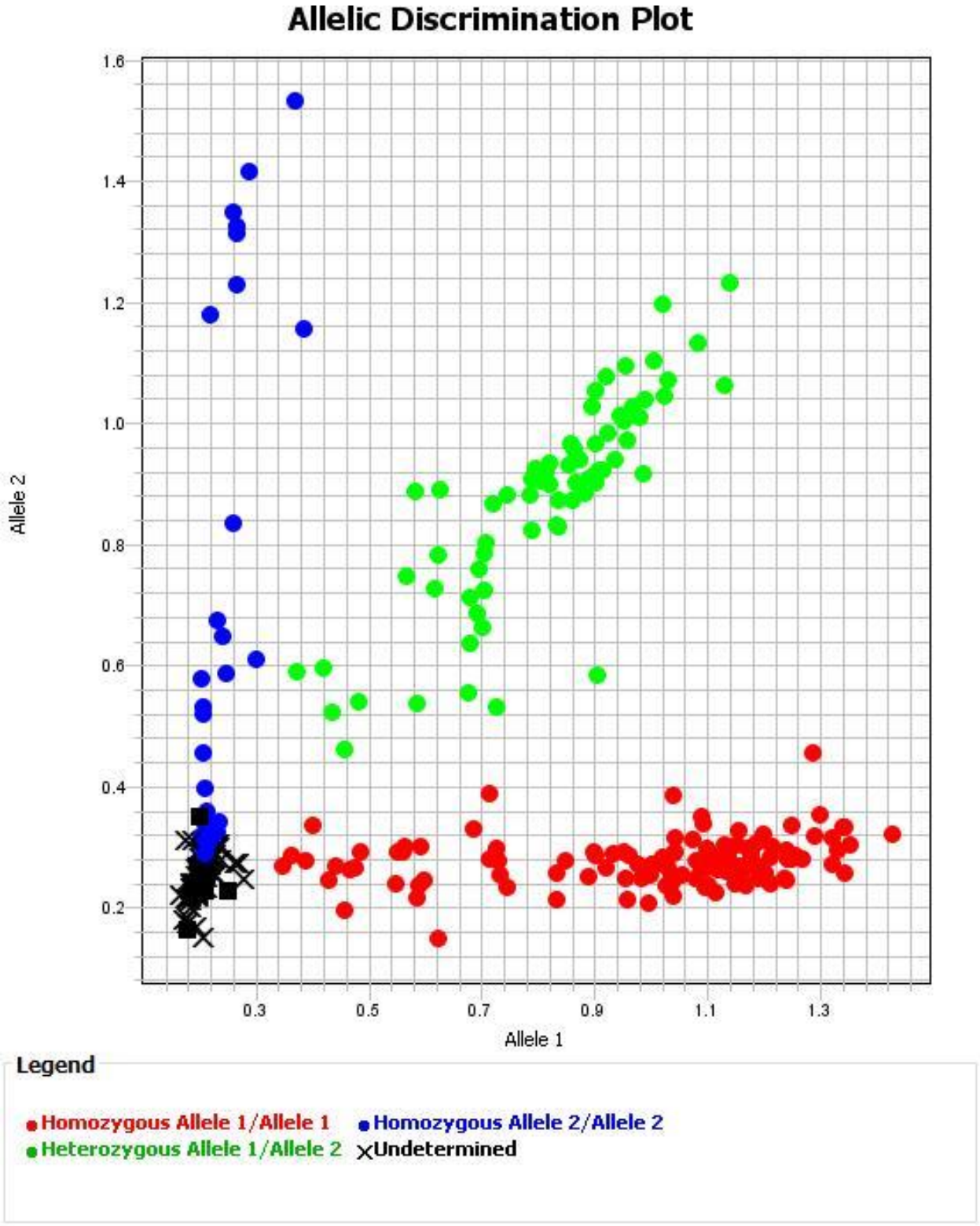

Figure 8: Plate 5-8 with SNP rs1143634.

The following graphs allowed access to determine patients homozygous and heterozygous alleles. The subjects were separated based on Homozygous Allele 1/ Allele 1, Heterozygous Allele 1/ Allele 2, and Homozygous Allele 2/ Allele 2.

4.0 Statistical Summary

The following statistical analysis was performed using GraphPad Software. It provides a summary of statistics between different groups and their genotypes.

Table 24: Chi-square test comparing the genotypes of Group A with Group B after genotyping with the SNP rs1800587:

GENOTYPES	GROUP A	GROUP B
GG	29	70
AA	17	25
GA	24	74

Chi-square, df	**3.628, 2**
P value	**0.1630**
P value summary	**ns**
One- or two-sided	**NA**
Statistically significant (P < 0.05)?	**No**

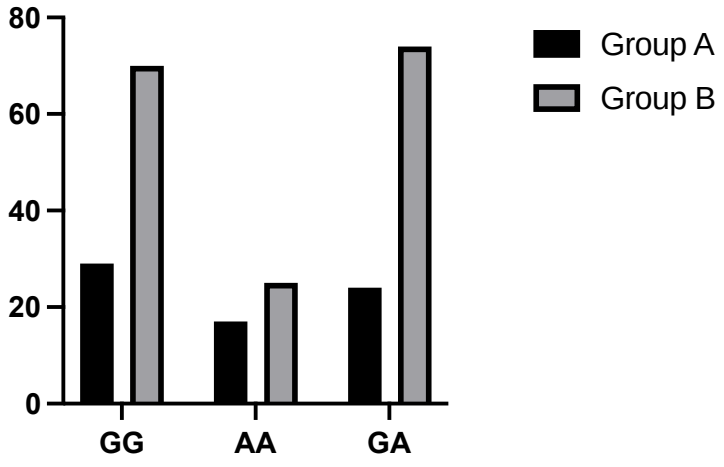

Figure 9: Genotypes of Group A Vs Group B with SNP rs1800587

Table 25: Chi-square test comparing the genotypes of Group A with Group C after genotyping with the SNP rs1800587.

GENOTYPES	GROUP A	GROUP C
GG	29	115
AA	17	28
GA	24	110

Chi-square, df	**8.193, 2**
P value	**0.0166**
P value summary	*****
One- or two-sided	**NA**
Statistically significant (P < 0.05)?	**Yes**

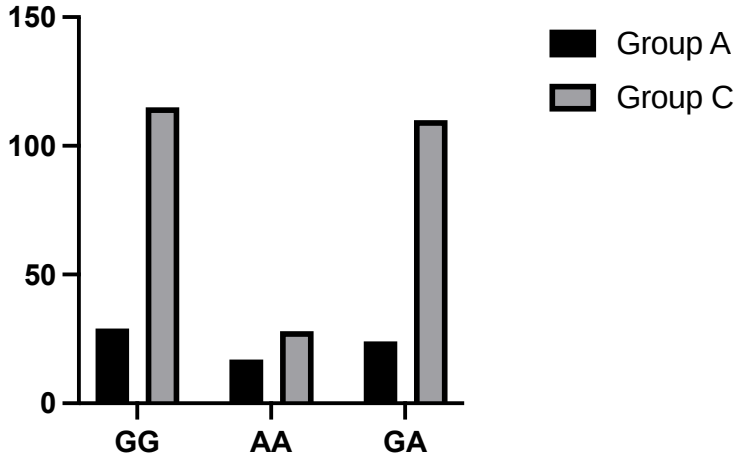

Figure 10: Genotypes of Group A Vs Group C with SNP rs1800587.

Table 26: Chi-square test comparing the genotypes of Group A with Group D after genotyping with the SNP rs1800587.

GENOTYPES	GROUP A	GROUP D
GG	29	39
AA	17	13
GA	24	34

Chi-square, df	**2.109,2**
P value	**0.3483**
P value summary	**ns**
One- or two-sided	**NA**
Statistically significant (P < 0.05)?	**No**

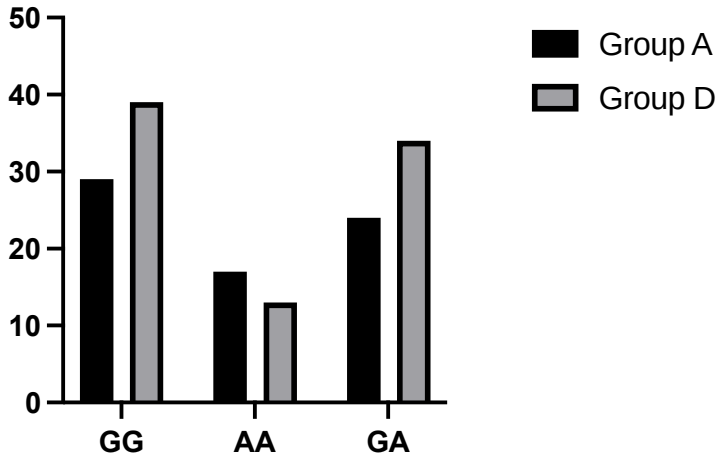

Figure 11: Genotypes of Group A Vs Group D with SNP rs1800587

In this study, the chi-square test results comparing Group A with Group B, C, and D after genotyping them with SNP rs1800587(*IL-1a*) showed that *IL-1a* was significantly (P<0.0166) associated in subjects with Periodontitis (Group C). However, it was not associated with hypertension.

Table 27: Chi-square test comparing the genotypes of Group A with Group B after genotyping with the SNP rs1143634.

GENOTYPES	GROUP A	GROUP B
GG	39	56
AA	12	10
GA	19	103

Chi-square, df	**24.20, 2**
P value	**<0.0001**
P value summary	********
One- or two-sided	**NA**
Statistically significant (P < 0.05)?	**Yes**

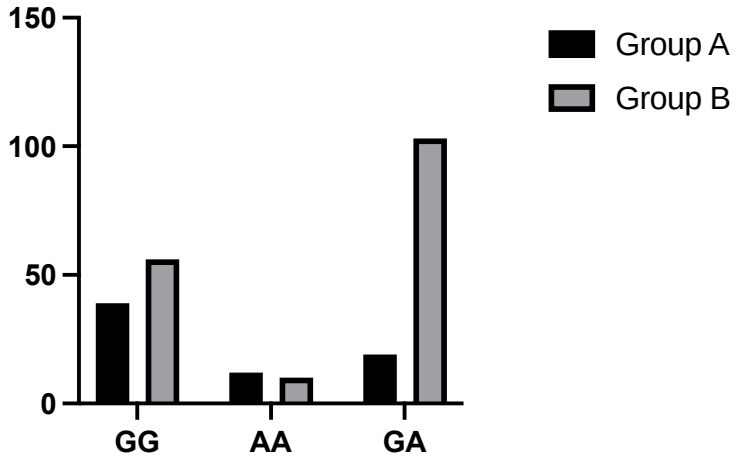

Figure 12: Genotypes of Group A Vs Group B with SNP rs1143634.

Table 28: Chi-square test comparing the genotypes of Group A with Group C after genotyping with the SNP rs1143634.

GENOTYPES	GROUP A	GROUP C
GG	39	157
AA	12	19
GA	19	77

Chi-square, df	**5.864, 2**
P value	**0.0533**
P value summary	**ns**
One- or two-sided	**NA**
Statistically significant (P < 0.05)?	**No**

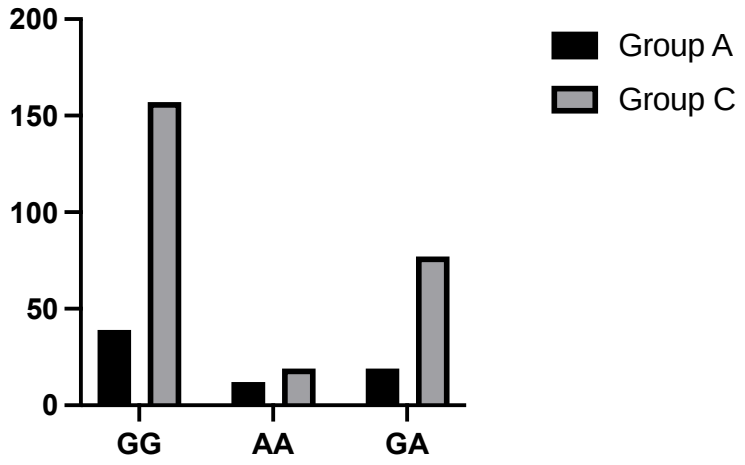

Figure 13: Genotypes of Group A Vs Group C with SNP rs1143634.

Table 29: Chi-square test comparing the genotypes of Group A with Group D after genotyping with the SNP rs1143634.

GENOTYPES	GROUP A	GROUP D
GG	39	50
AA	12	15
GA	19	31

Chi-square, df	**0.5132, 2**
P value	**0.7737**
P value summary	**ns**
One- or two-sided	**NA**
Statistically significant (P < 0.05)?	**No**

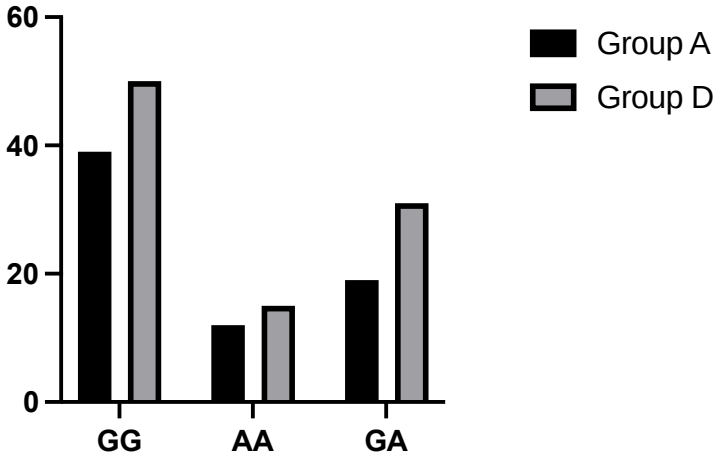

Figure 14: Genotypes of Group A Vs Group D with SNP rs1143634.

In this study, the chi-square test results comparing Group A with Group B, C, and D after genotyping them with SNP rs1143634(*IL-1b*) showed that *IL-1b* was significantly (P<0.0001) associated in subjects with both Periodontitis and Hypertension (Group B).

Table 30: Chi-square test comparing the genotype of male & female subjects from Group A when genotyped with rs1800587 (*IL-1a*).

GENOTYPES	MALE	FEMALE
GG	17	15
AA	3	6
GA	13	16

Chi-square, df	**1.211, 2**
P value	**0.5459**
P value summary	**ns**
One- or two-sided	**NA**
Statistically significant (P < 0.05)?	**No**

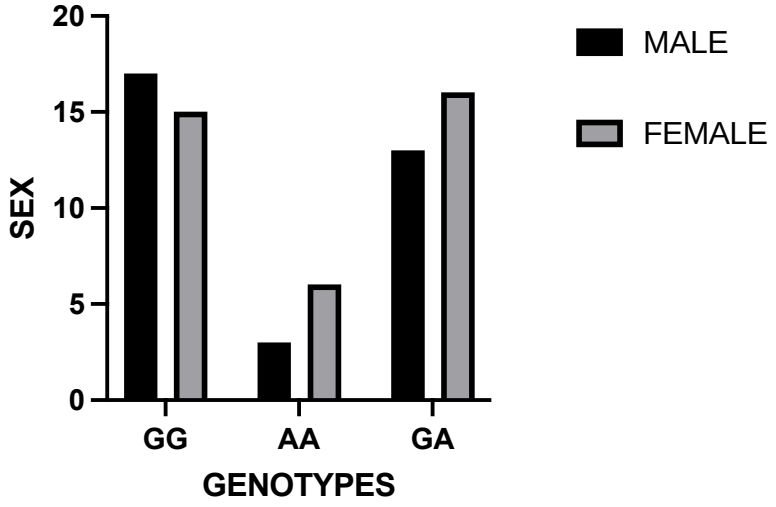

Figure 15: Genotypes of males and females of Group A with SNP rs1800587.

Table 31: Chi-square test comparing the genotype of male & female subjects from group B when genotyped with rs1800587 (*IL-1a*).

GENOTYPES	MALES	FEMALES
GG	41	33
AA	11	13
GA	41	30

Chi-square, df	**1.216, 2**
P value	**0.5444**
P value summary	**ns**
One- or two-sided	**NA**
Statistically significant (P < 0.05)?	**No**

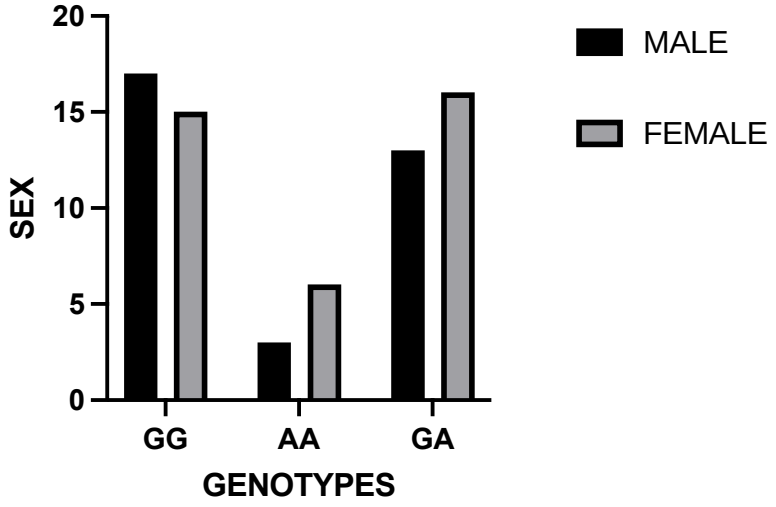

Figure 16: Genotypes of males and females of Group A with SNP rs1800587.

Table 32: Chi-square test comparing the genotype of male & female subjects from group C when genotyped with rs1800587 (*IL-1a*).

GENOTYPES	MALES	FEMALES
GG	77	51
AA	15	16
GA	44	50

Chi-square, df	**4.695, 2**
P value	**0.0956**
P value summary	**ns**
One- or two-sided	**NA**
Statistically significant (P < 0.05)?	**No**

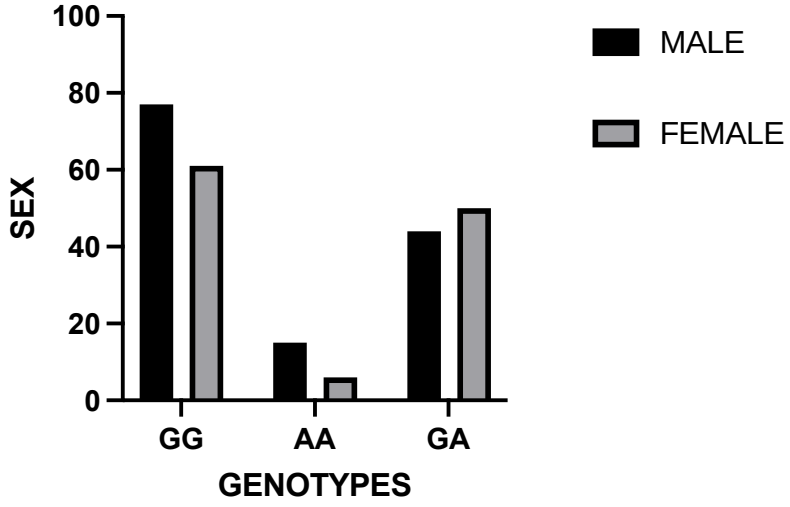

Figure 17: Genotypes of males and females of Group C with SNP rs1800587.

Table 33: Chi-square test comparing the genotype of male & female subjects from group D when genotyped with rs1800587 (*IL-1a*).

GENOTYPES	MALES	FEMALES
GG	19	26
AA	1	6
GA	14	20

Chi-square, df	**2.041, 2**
P value	**0.3604**
P value summary	**ns**
One- or two-sided	**NA**
Statistically significant (P < 0.05)?	**No**

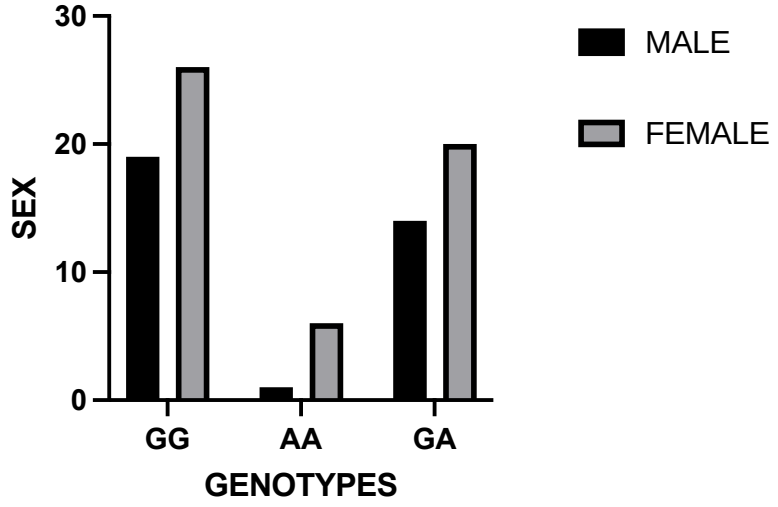

Figure 18: Genotypes of males and females of Group D with SNP rs1800587.

Table 34: Chi-square test comparing the genotype of male & female subjects from group A when genotyped with rs1143634 (*IL-1b*).

GENOTYPES	MALES	FEMALES
GG	20	19
AA	5	6
GA	8	12

Chi-square, df	**0.6902, 2**
P value	**0.7081**
P value summary	**ns**
One- or two-sided	**NA**
Statistically significant (P < 0.05)?	**No**

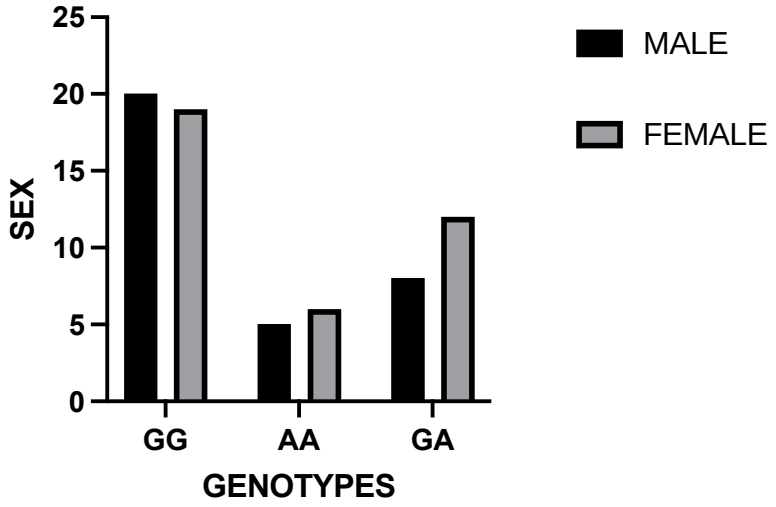

Figure 19: Genotypes of males and females of Group A with SNP rs1143634.

Table 35: Chi-square test comparing the genotype of male & female subjects from group B when genotyped with rs1143634 (*IL-1b*).

GENOTYPES	MALES	FEMALES
GG	53	52
AA	7	3
GA	33	21

Chi-square, df	**2.592, 2**
P value	**0.2736**
P value summary	**ns**
One- or two-sided	**NA**
Statistically significant (P < 0.05)?	**No**

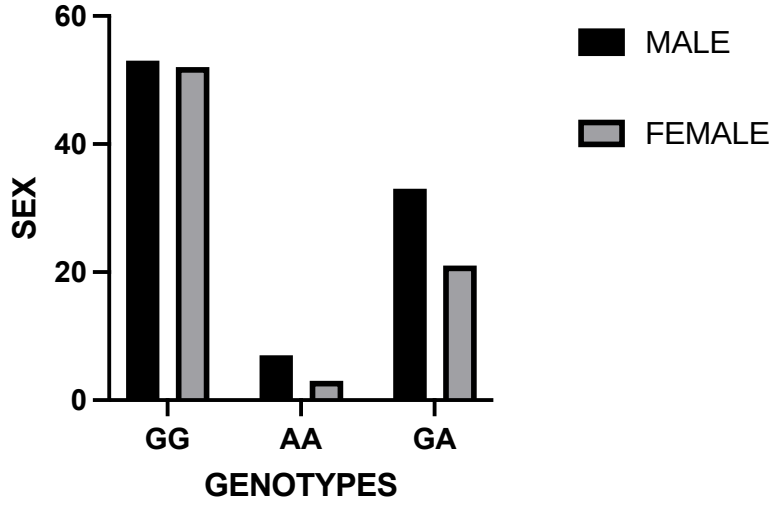

Figure 20: Genotypes of males and females of Group B with SNP rs1143634.

Table 36: Chi-square test comparing the genotype of male & female subjects from group C when genotyped with rs1143634 (*IL-1b*).

GENOTYPES	MALES	FEMALES
GG	82	75
AA	12	7
GA	42	35

Chi-square, df	**0.8421, 2**
P value	**0.6563**
P value summary	**ns**
One- or two-sided	**NA**
Statistically significant (P < 0.05)?	**No**

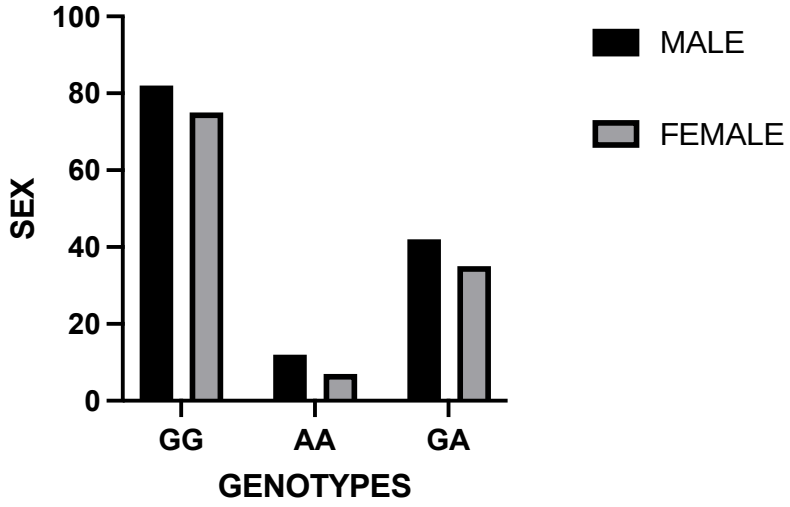

Figure 21: Genotypes of males and females of Group C with SNP rs1143634.

Table 37: Chi-square test comparing the genotype of male & female subjects from group D when genotyped with rs1143634 (*IL-1b*).

GENOTYPES	MALES	FEMALES
GG	23	30
AA	4	5
GA	7	17

Chi-square, df	**1.501, 2**
P value	**0.4722**
P value summary	**ns**
One- or two-sided	**NA**
Statistically significant (P < 0.05)?	**No**

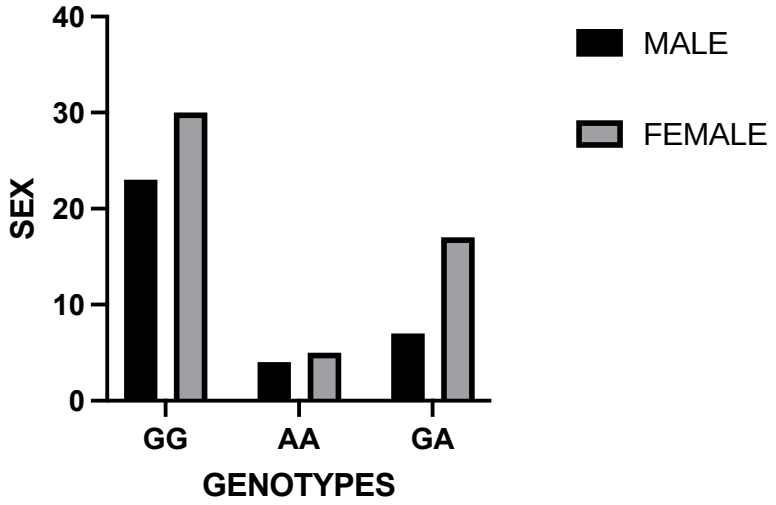

Figure 22: Genotypes of males and females of Group D with SNP rs1143634.

Table 38: Chi-square test to compare the oral hygiene status of Group A with Group B.

GROUP	IMPROVED	WORSENED	NEUTRAL
GROUP A	28	30	12
GROUP B	100	63	6

Chi-square, df	**15.94, 2**
P value	**0.0003**
P value summary	*******
One- or two-sided	**NA**
Statistically significant (P < 0.05)?	**Yes**

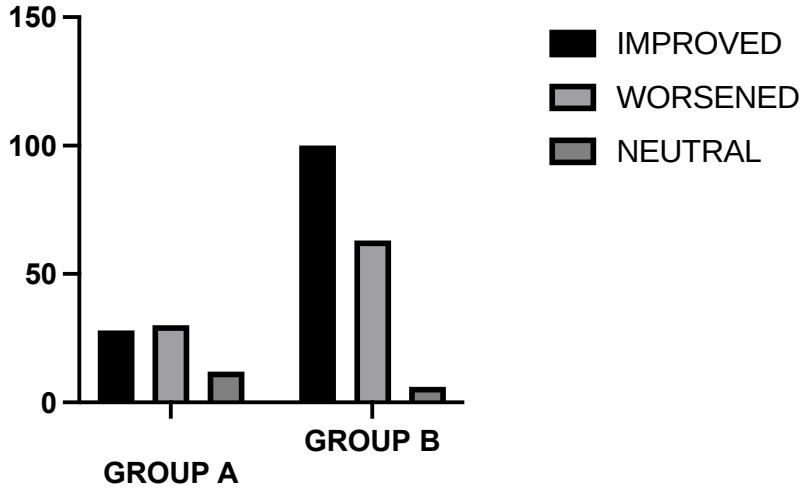

Figure 23: Oral hygiene status of Group A with Group B.

Table 39: Chi-square test to compare the oral hygiene status of Group A with Group C.

GROUP	IMPROVED	WORSENED	NEUTRAL
GROUP A	28	30	12
GROUP C	151	99	3

Chi-square, df	**34.09, 2**
P value	**<0.0001**
P value summary	********
One- or two-sided	**NA**
Statistically significant (P < 0.05)?	**Yes**

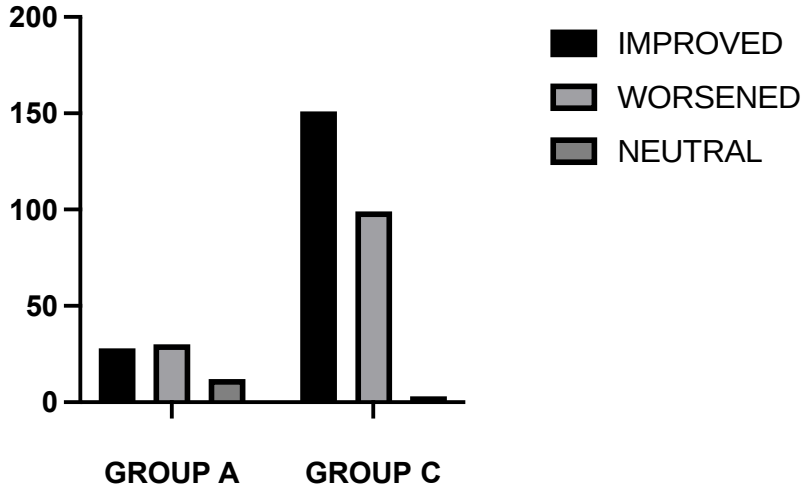

Figure 24: Oral hygiene status of Group A with Group C.

Table 40: Chi-square test to compare the oral hygiene status of Group A with Group D.

GROUP	IMPROVED	WORSENED	NEUTRAL
GROUP A	28	30	12
GROUP D	51	29	6

Chi-square, df	**7.147, 2**
P value	**0.0281**
P value summary	*****
One- or two-sided	**NA**
Statistically significant (P < 0.05)?	**Yes**

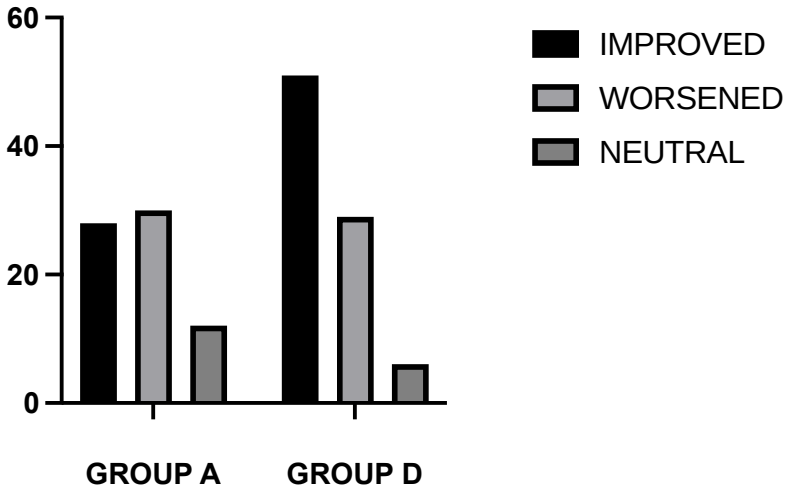

Figure 25: Oral hygiene status of Group A with Group D.

Table 41: Comparing genotypes among the subjects who have Improved in Group A with Group B after genotyping with rs1800587.

GROUP	GG	GA	AA
A	17	12	6
B	46	48	12

Chi-square, df	**1.374, 2**
P value	**0.5030**
P value summary	**ns**
One- or two-sided	**NA**
Statistically significant (P < 0.05)?	**No**

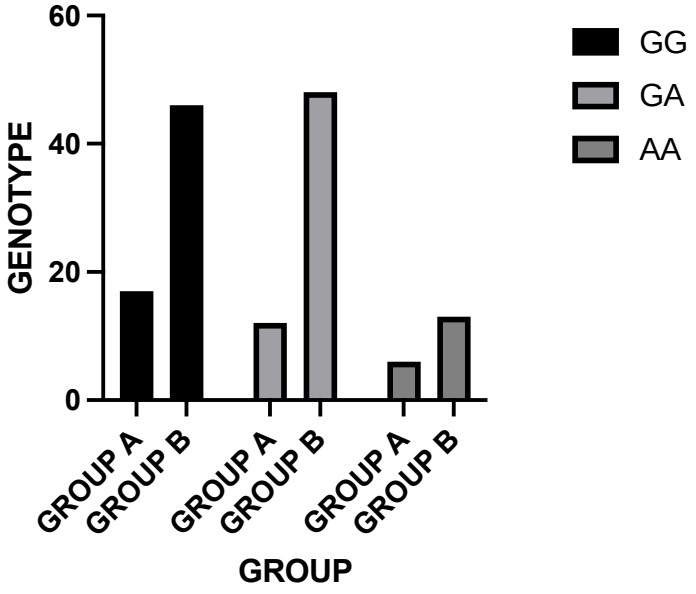

Figure 26: Comparing the genotypes of the SNP rs1800587 from Group A with Group B in relation to their Improved OHS.

Table 42: Comparing genotypes among the subjects who have Improved in Group A with Group C after genotyping with rs1800587.

GROUP	GG	GA	AA
A	17	12	6
C	63	58	9

Chi-square, df	**3.862, 2**
P value	**0.1450**
P value summary	**ns**
One- or two-sided	**NA**
Statistically significant (P < 0.05)?	**No**

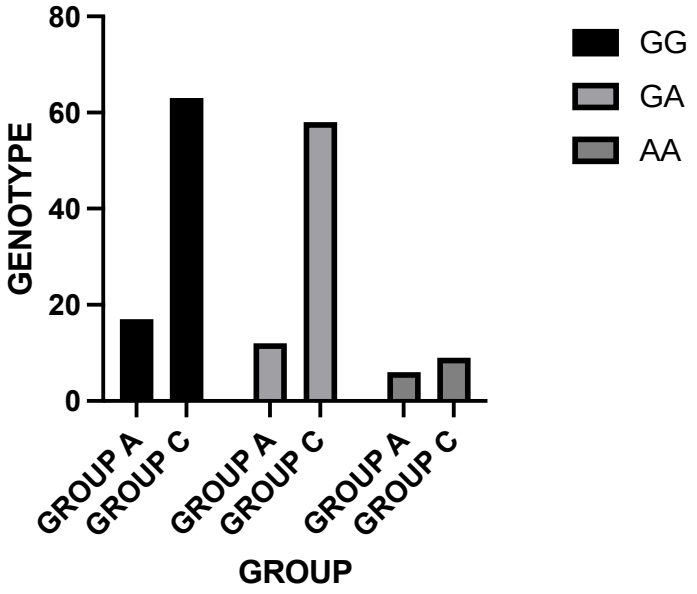

Figure 27: Comparing the genotypes of the SNP rs1800587 from Group A with Group C in relation to their Improved OHS.

Table 43: Comparing genotypes among the subjects who have Improved in Group A with Group D after genotyping with rs1800587.

GROUP	GG	GA	AA
A	17	12	6
D	13	14	2

Chi-square, df	**2.144, 2**
P value	**0.3424**
P value summary	**ns**
One- or two-sided	**NA**
Statistically significant (P < 0.05)?	**No**

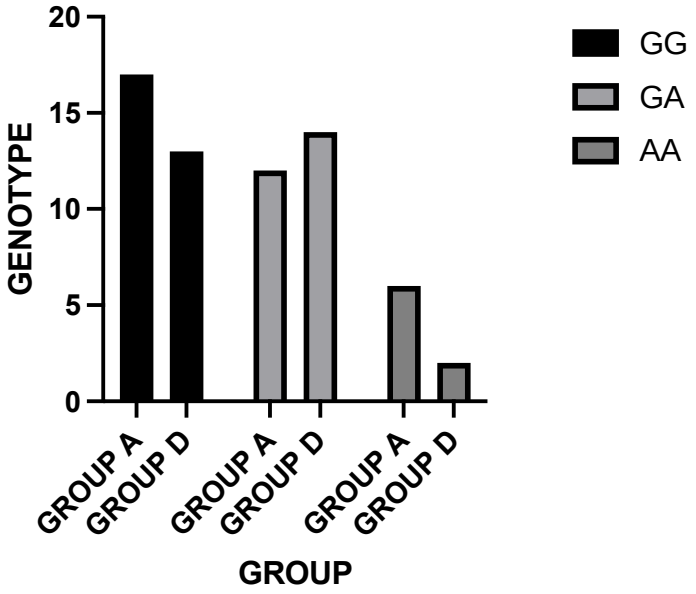

Figure 28: Comparing the genotypes of the SNP rs1800567 from Group A with Group D in relation to their Improved OHS.

Table 44: Comparing genotypes among the subjects who Worsened in Group A with the Group B after genotyping with rs1800587.

GROUP	GG	GA	AA
A	7	12	3
B	22	21	5

Chi-square, df	**1.225, 2**
P value	**0.5420**
P value summary	**ns**
One- or two-sided	**NA**
Statistically significant (P < 0.05)?	**No**

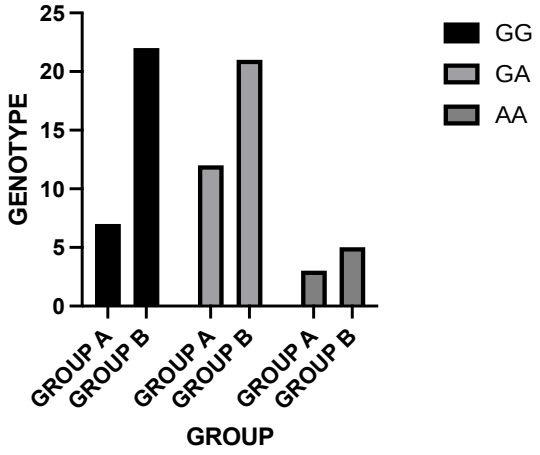

Figure 29: Comparing the genotypes of the SNP rs1800587 from Group A with Group B in relation to their worsened OHS.

Table 45: Comparing genotypes among the subjects who have Worsened in Group A with the Group C after genotyping with rs1800587.

GROUP	GG	GA	AA
A	7	12	3
C	36	29	10

Chi-square, df	**2.021, 2**
P value	**0.3641**
P value summary	**ns**
One- or two-sided	**NA**
Statistically significant (P < 0.05)?	**No**

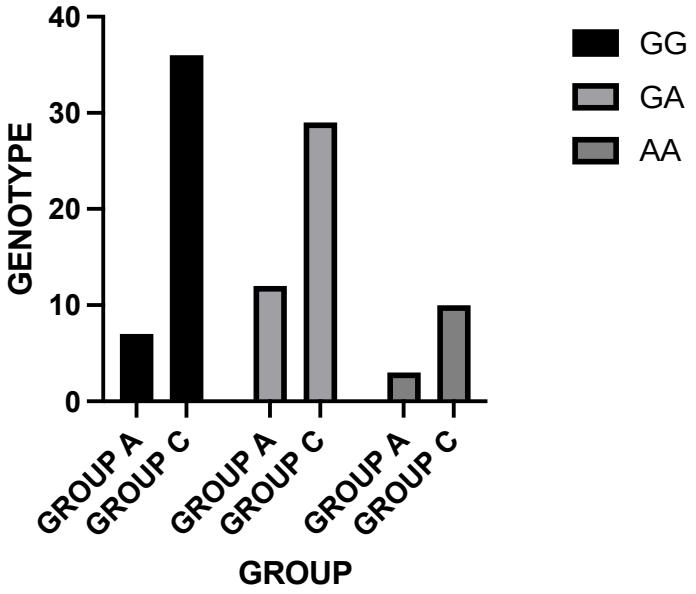

Figure 30: Comparing the genotypes of the SNP rs1800567 from Group A with Group C in relation to their worsened OHS.

Table 46: Comparing genotypes among the subjects who have Worsened in Group A with Group D after genotyping with rs1800587.

GROUP	GG	GA	AA
A	7	12	3
D	18	10	4

Chi-square, df	**3.430, 2**
P value	**0.1799**
P value summary	**ns**
One- or two-sided	**NA**
Statistically significant (P < 0.05)?	**No**

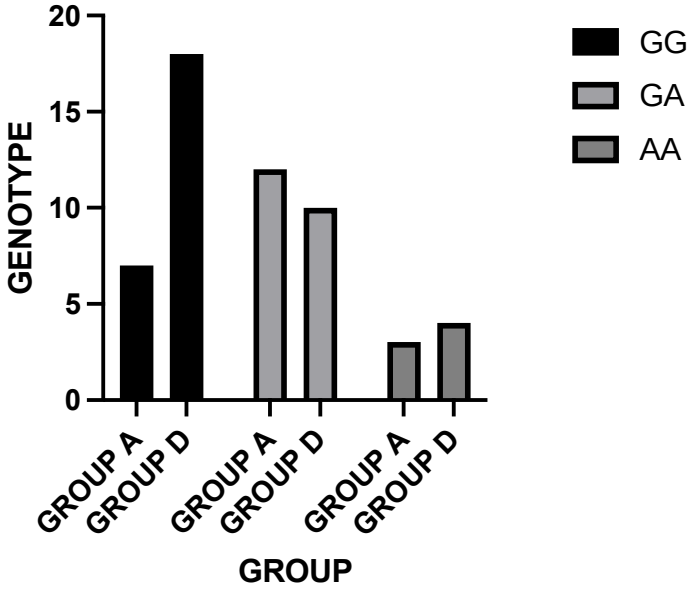

Figure 31: Comparing the genotypes of the SNP rs1800567 from Group A with Group D in relation to their worsened OHS.

Table 47: Comparing genotypes among the subjects who have Improved in Group A with Group B after genotyping with rs1143634.

GROUP	GG	GA	AA
A	21	6	8
B	69	33	4

Chi-square, df	**13.23, 2**
P value	**0.0013**
P value summary	******
One- or two-sided	**NA**
Statistically significant (P < 0.05)?	**Yes**

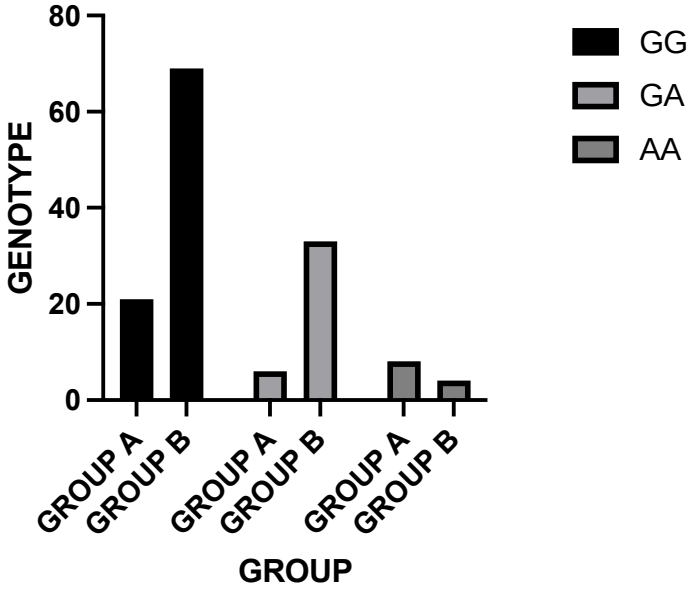

Figure 32: Comparing the genotypes of the SNP rs1143634 from Group A with Group B in relation to their improved OHS.

Table 48: Comparing genotypes among the subjects who have Improved in Group A with Group C after genotyping with rs1143634.

GROUP	GG	GA	AA
A	21	6	8
C	83	41	8

Chi-square, df	**10.09, 2**
P value	**0.0065**
P value summary	******
One- or two-sided	**NA**
Statistically significant (P < 0.05)?	**Yes**

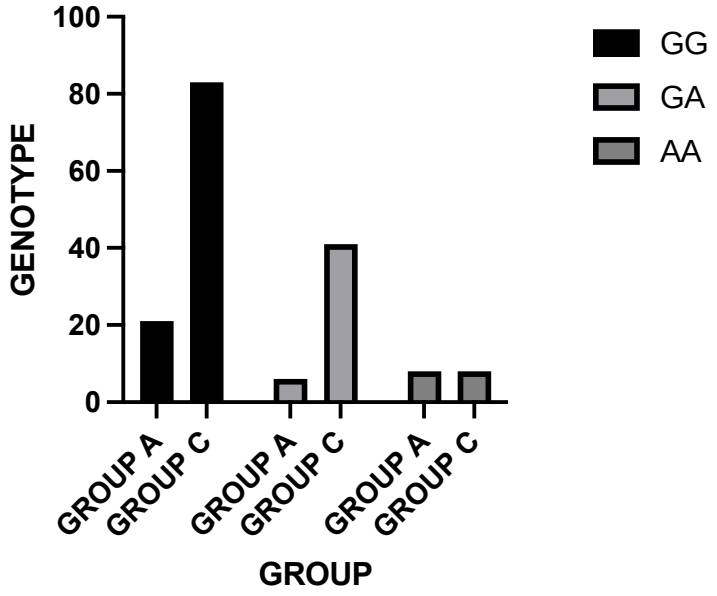

Figure 33: Comparing the genotypes of the SNP rs1143634 from Group A with Group C in relation to their improved OHS.

Table 49: Comparing genotypes among the subjects who have Improved in Group A with Group D after genotyping with rs1143634.

GROUP	GG	GA	AA
A	21	6	8
D	16	10	2

Chi-square, df	**4.554, 2**
P value	**0.1026**
P value summary	**ns**
One- or two-sided	**NA**
Statistically significant (P < 0.05)?	**No**

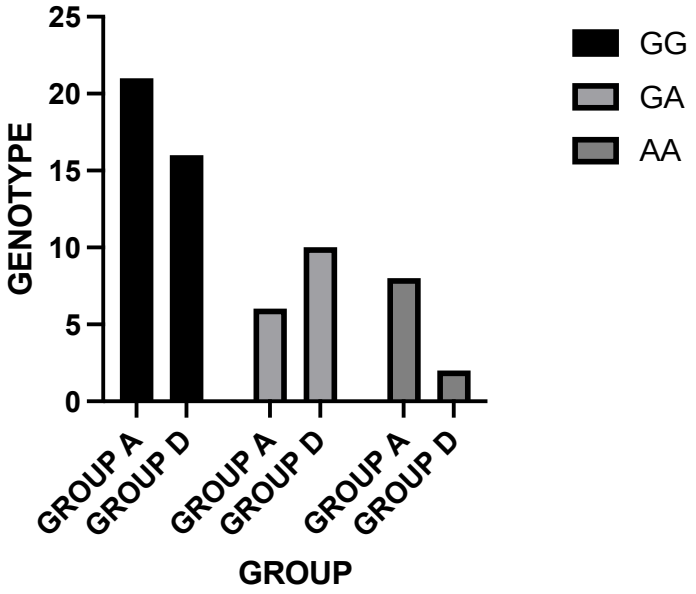

Figure 34: Comparing the genotypes of the SNP rs1143634 from Group A with Group D in relation to their improved OHS.

Table 50: Comparing genotypes among the subjects who have Worsened in Group A with the Group B after genotyping with rs1143634.

GROUP	GG	GA	AA
A	11	10	1
B	29	17	2

Chi-square, df	**0.6856, 2**
P value	**0.7098**
P value summary	**ns**
One- or two-sided	**NA**
Statistically significant (P < 0.05)?	**No**

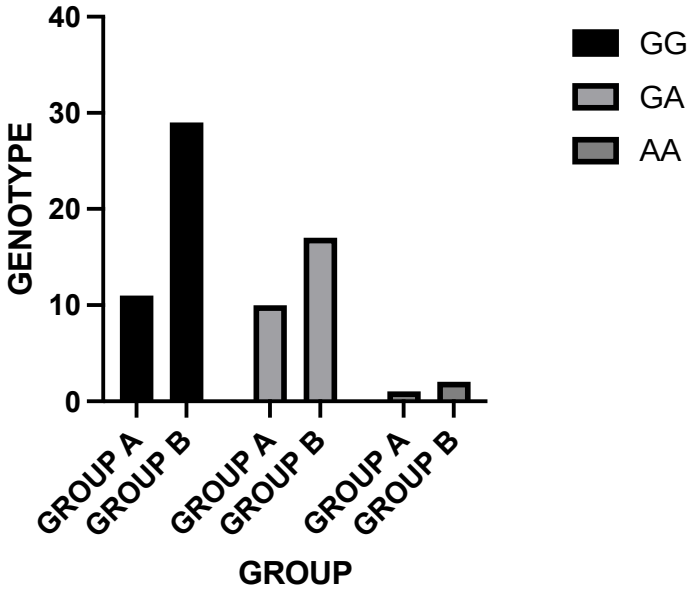

Figure 35: Comparing the genotypes of the SNP rs1143634 from Group A with Group B in relation to their worsened OHS.

Table 51: Comparing genotypes among the subjects who have Worsened in Group A with Group C after genotyping with rs1143634.

GROUP	GG	GA	AA
A	11	10	1
C	52	29	6

Chi-square, df	**1.162, 2**
P value	**0.5593**
P value summary	**ns**
One- or two-sided	**NA**
Statistically significant (P < 0.05)?	**No**

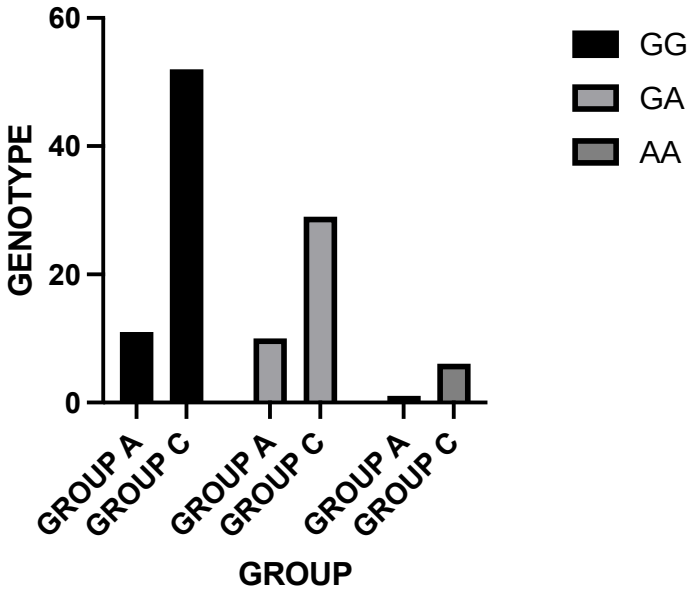

Figure 36: Comparing the genotypes of the SNP rs1143634 from Group A with Group C in relation to their worsened OHS.

Table 52: Comparing genotypes among the subjects who have Worsened in Group A with Group D after genotyping with rs1143634.

GROUP	GG	GA	AA
A	11	10	1
D	23	9	0

Chi-square, df	**3.558, 2**
P value	**0.1688**
P value summary	**ns**
One- or two-sided	**NA**
Statistically significant (P < 0.05)?	**No**

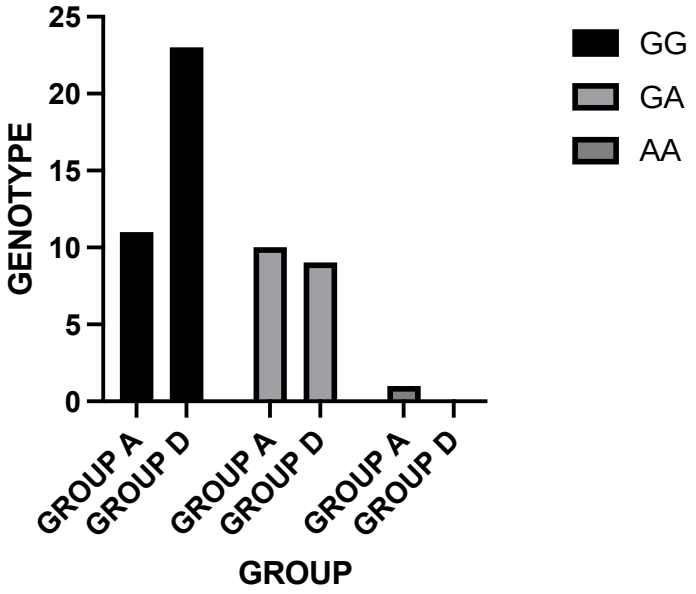

Figure 37: Comparing the genotypes of the SNP rs1143634 from Group A with Group D in relation to their worsened OHS.

5.0 Discussion

Periodontitis is one of the most chronic infectious conditions that cause systemic inflammation. Most systemic inflammatory conditions lead to endothelial dysfunction. The most common type of systemic inflammation is Cardiovascular Disorders (CVD). Several studies have suggested a possible association between periodontal diseases and high blood pressure. Increased systemic inflammation is associated with high levels of C-reactive proteins (CRP) (Tsakos et al., 2010). The C- reactive proteins (CRP) levels can predict the development of hypertension. The other inflammatory markers, like the IL-6 and TNF- alpha, are elevated in patients with periodontitis compared to the healthy subjects (Macedo Paizan & Vilela-Martin, 2014). In the present study, we have included subjects with periodontitis and hypertension based on their plaque score and medical drug history, respectively. We analyzed the association of PD with HBP and PD without HBP in the subjects to understand the association of hypertension in subjects with periodontitis. We have genotyped the genomic DNA of the subjects using SNPs rs1800587(*IL-1a*) and rs1143634 (*IL-1b*) to analyze the influence of the genes on PD and the treatment outcomes.

In a study, biomarkers of periodontal inflammation were compared before and after treatment. An early increase in the levels of inflammatory mediators and BP, as well as endothelial dysfunction, were observed shortly after treatment. However, six months later, there was a significant improvement in the periodontal variables. (Del Pinto et al., 2020). Furthermore, the multifactorial etiology of both periodontitis and CVD and the fact that both share a common inflammatory nature indicates that removing the local gingival inflammation may not be beneficial. Some possible biomarkers associated with periodontitis must be examined for future risk of atherosclerosis (D'Aiuto, Orlandi, & Gunsolley, 2013). In the present study, we assessed

the periodontal status of the subjects by comparing their plaque score before and after non-surgical therapy within three months to two years. There was a significant improvement in the plaque score in the subjects with the IL-1b gene having PD and HBP (Group B) and in subjects with PD but without HBP (Group C). Therefore, according to the present study, subjects with PD, with or without HBP, significantly improved their plaque score after non-surgical therapy. Also, we have assessed the association of the genetic marker, Interleukin-1 in subjects with PD and HBP. The chi-square test results comparing Group A with Group B, C, and D after genotyping them with SNP rs1800587 (*IL-1a*) showed that *IL-1a* was significantly (P<.0166) associated in subjects with PD (Group C). However, it was not associated with HBP. In this study, the chi-square test results comparing Group A with Group B, C, and D after genotyping them with SNP rs1143634 (*IL-1b*) showed that *IL-1b* was significantly (P<.0001) associated in subjects with both PD and HBP (Group B).

A study focusing on non-surgical periodontal therapy also supported the association between periodontitis and hypertension. Improvement of periodontal status following intensive periodontal therapy was directly related to the 24-hour improvement of blood pressure. These effects on blood pressure were accompanied by reduced systemic pro-hypertensive cytokine levels (IL-6, IL-17A.IFN-gamma, TNF-alpha) (Czesnikiewicz-Guzik et al., 2019). In the present study, we assessed the plaque score of the patients before and after non-surgical periodontal therapy. The chi-square tests performed to compare the oral hygiene status of the healthy group without PD and HBP (Group A) with Group B, C, and D have significantly improved their plaque score (p =0.0003, 0.0001, 0.02, respectively). There was a significant improvement in the plaque score in subjects with PD with or without HBP.

A positive association between periodontitis and hypertension was seen in a systemic analysis. Patients with moderate and severe periodontitis have 20% odds of hypertension than those without periodontitis. The more severe periodontitis is, the higher the likelihood of having hypertension (Muñoz Aguilera et al., 2020). However, in the present study, the prevalence of periodontitis is equal among the subjects with or without hypertension. The genetic marker, Interleukin-1, did not show any significant sex predilection.

In the present study, we evaluate the subjects based on their plaque score before and after the non-surgical therapy. The healthy dental plaque biofilm predominantly comprises commensal, non-pathogenic microbial members. However, these commensal members are not mute counterparts, and they constantly communicate with the other bacterial species and the host tissues. They also provide colonization resistance against pathogenic and harmful organisms. The commensals initiate a signal cascade that converges the message of tolerance, whereas pathogenic bacteria induce a robust host inflammatory response. Eventually, it produces pro-inflammatory cytokines in the oral epithelial cells at a low level, which causes the expression of E-selectin in the vascular endothelial tissues and the establishment of various cytokine gradients (Seneviratne et al., 2011). The present study analyzed the association between Interleukin-1 and periodontal disease. We have seen a significant association between Interleukin -1 (*IL-1a* and *IL-1b*) and subjects with periodontitis. The study showed that *IL-1a* was associated with subjects with only periodontitis and not with hypertension. However, *IL-1b* was significantly associated with subjects with both periodontitis and hypertension. Also, it has been confirmed in a study that individuals positive for the *IL-1* genotype were more likely to be colonized by high levels of periodontal pathogens. The overexpression of IL-1a and IL-1b in response to organisms in subgingival plaque may increase gingival inflammation and subgingival plaque (Grigoriadou et al., 2010). However, in the present

study, non-surgical periodontal therapy caused a significant reduction in the plaque score in subjects carrying the common allele for *IL-1a* and *IL-1b*.

In a study, complex patterns of response to oral hygiene instructions were evaluated in 227 periodontal patients using longitudinal plaque and bleeding index data recorded during multiple visits. The subjects included in this study had three types of oral hygiene statuses improved, worsened, and fluctuating based on their response to oral hygiene instructions. These oral hygiene patterns were based on the plaque and gingival bleeding index considering the age, sex, ethnic background, interleukin-1 alpha, beta genotypes, diabetes status, smoking habits, and another concomitant disease. Of the several variables considered, only ethnic background showed a statistically significant difference ($p<0.05$). The white individuals, more often than other ethnic groups, fluctuated regarding oral hygiene quality after instructions (Amoo-Achampong, Vitunac, Deeley, Modesto, & Vieira, 2018). In the present study, the oral hygiene status of subjects having periodontitis was evaluated considering risk factors like hypertension and pro-inflammatory cytokines like Interleukin-1. We also evaluated the possible role of *IL-1a* and *IL-1b* on the oral hygiene status of the patients receiving non-surgical therapy and oral hygiene instructions. The plaque index of 578 subjects was assessed from two different appointments, and the subjects were divided into three categories based on their oral hygiene status improved, worsened, and neutral. IL-1a was only associated with subjects with PD, and *IL-1b* was associated with subjects with both PD and HBP. However, there was no sex predominance for *IL-1*. Also, in this study, when comparing the genotypes of the subjects having PD and HBP (Group B and C) with *IL-1b* with the unaffected group (Group A), there was a significant improvement in Oral hygiene status following non-surgical intervention and strictly following oral hygiene instructions.

The finding that a specific genotype in the *IL-1* gene cluster correlates with severe periodontitis suggests a genetic mechanism by which some individuals if subjected to high bacterial accumulations, might have a more vigorous immune-inflammatory response leading to severe periodontitis. It is notable from both studies that *IL-1b* polymorphisms are associated with severe periodontitis, and this correlates with *IL-1 b* production rates (Kornman et al., 1997). In the present study, IL-1b was predominantly associated with subjects with PD and HBP (Group B).

The Interleukin-1 gene polymorphisms, most prominently *IL-1a* and *IL-1b*, and their association to cause chronic periodontitis in the white population revealed that the *IL-1* genotype had no effect in six of the eight groups where the sample size was smaller (n< 200). However, when the sample size was more significant (n>200) in the last two groups, there was a statistically significant association between the genetic markers and disease (Karimbux et al., 2012). Also, a study comparing the oral hygiene status of subjects with different ethnicities showed that the white population had more fluctuations in their oral hygiene status than the other individuals included in the study (Amoo-Achampong, Vitunac, Deeley, Modesto, & Vieira, 2018). The subjects included in the present study had diverse ethnicity. Therefore, the research did not target any specific group of the population.

In a study conducted by (McFarlane & Meikle, 1991), it was demonstrated that IL-2, IL-2R, and IL-4 could be detected in sera of periodontitis patients at significantly higher levels than those found in control subjects. Also, compared to the previous study on the *IL-1 b* gene, there is no correlation between the degree of bone loss or pocket formation clinically. It is likely to be expected as the pocket depth represents the cumulative effects of disease activity. Longitudinal studies would be more helpful in confirming whether clinical indicators of disease activity correlate with the laboratory values of various cytokines. In the present study, we have analyzed

the association of the *IL-1a* and *IL-1b* genes with periodontitis and hypertension. This study showed a significant association of *IL-1a* in subjects with periodontitis, and *IL-1b* was associated with subjects with periodontitis with hypertension.

6.0 Conclusion

1. It is one of the first studies to study the association of the interleukin-1 gene in subjects with periodontitis and hypertension and to assess the oral hygiene status of these subjects following non-surgical interventions and instructions.
2. Previous studies have assessed the role of *IL-1a* and *IL-1b* gene polymorphisms in increasing the susceptibility to periodontitis and hypertension. The present study used SNPs rs1800587 and rs1143634 to study the association of these SNPs in subjects with periodontitis and hypertension.
3. We concluded that *IL-1a* gene polymorphisms are significantly associated with periodontitis, and *IL-1b* gene polymorphisms are associated with both periodontitis and hypertension without sex predilection.
4. Also, all the subject's oral hygiene status showed a significant improvement in their plaque score irrespective of the genotype of the *IL-1* gene. But only *IL-1b* genotypes of the subjects from Group B and Group C have shown a significant improvement of oral hygiene status following non-surgical therapy.

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