A COMPARATIVE STUDY OF SYNBIOTIC AS AN ADD ON THERAPY TO STANDARD TREATMENT IN PATIENTS WITH AGGRESSIVE PERIODONTITIS

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CERTIFICATE

This is to certify that the dissertation entitled, **"A COMPARATIVE STUDY OF SYNBIOTIC AS AN ADD ON THERAPY TO STANDARD TREATMENT IN PATIENTS WITH AGGRESSIVEPERIODONTITIS"** submitted by DR.M.GANGADEVI, in partial fulfillment for the award of the degree of Doctor of Medicine in Pharmacology by The TamilnaduDr.M.G.R.Medical University, Chennai is a Bonafide record of the work done by herin the Institute of Pharmacology, Madras Medical College during the academic year 2013-16.

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I Dr.M.Gangadevi solemnly declared that the dissertation entitled, **"A COMPARATIVE STUDY OF SYNBIOTIC AS AN ADD ON THERAPY TO STANDARD TREATMENT IN PATIENTS WITH AGGRESSIVE PERIODONTITIS"** has been prepared by me. This is submitted to The TamilnaduDr.M.G.R.Medical University, Chennai in partial fulfillment of the rules and regulations for M.D.degree examination in Pharmacology.

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TURNITIN ANTI-PLAGIARISM SOFTWARE – CERTIFICATE

CONTENTS

A COMPARATIVE STUDY OF SYNBIOTIC AS AN ADD ON THERAPY TO STANDARD TREATMENT IN PATIENTS WITH AGGRESSIVE PERIODONTITIS

ABSTRACT

AIM:

To evaluate the efficacy and safety of Synbiotic as an add on therapy to Standard treatment inthe management of patients with Aggressive Periodontitis compared to Standardtreatment alone.

METHODOLOGY

This was arandomized, prospective, placebo controlledstudy.60 patients with Aggressive Periodontitis wererandomized and blinded into two groups of 30 each. Control group received Scaling and Root planing [SRP] +Cap.Doxycycline 100mg twice daily for one week +Placebo one lozengetwice daily for 8 weeks andStudy group receivedScaling and Root planing [SRP] +Cap.Doxycycline 100mg twice daily for one week +Synbioticone lozenge twice daily for 8 weeks.The clinical parameters were recorded at baseline, 4,8 and 12 weeks.Oral hygiene index[OHI],Gingival bleeding index[GBI],Probing depth[PD] and Clinical attachment loss[CAL] were assessedat baseline and at the end of the study.

RESULTS

106patients were screened out of which 60 patients were included in the study.All patients completed the study and were included in analysis.On comparing the groups at the end of12weeks there was a statistically significant reduction ($P<0.01$) inOHI,GBI,PD & CALin Study group. No significant difference in the incidence of adverse events noted between the two groups.

CONCLUSION

Synbioticalong with standard therapy is highly efficacious in reducingOHI,GBI,PD & CALinpatients with Aggressive Periodontitis.

KEY WORDS

Synbiotic, Aggressive Periodontitis,Probing Depth,Clinical Attachment Loss.

INTRODUCTION

Periodontitis is one of the most common oral diseases with various incidence rates in different population. It is a chronic inflammatory disease of infectious origin leading to destruction of tooth-supporting tissues and is the major cause of tooth loss in young individuals[1].

The features of periodontitis include inflammatory changes in the gingiva, formation of pockets in the periodontal region, loss of connective tissues and alveolar bone around the affected tooth. If not treated properly loosening of tooth occurs and subsequently can lead on to loss of tooth[2].

Periodontitis can be classified into Aggressive periodontitis which is characterized by a rapid destruction of periodontal attachment and Chronicperiodontitis which is slowly progressive leading to gradual loss of attachment of supporting tissues.

Aggressive form of the disease commonly affects systemically healthy individuals less than 30 years old. It can be subdivided into Localized and Generalized aggressive periodontitis. The Localized form does not involvemore than two teeth. First molars and incisors are commonly affected. In Generalized form, at least three permanent teeth are involved other than first molars and incisors[3].

The etiology of Aggressive disease is very complex. Pathogenic bacteria are the primary etiologic agents in the pathogenesis of periodontitis. Although a number of bacteria are considered as the major etiologic agents of aggressive periodontitis, Aggregatibacter actinomycetemcomitans and Porphyromonas gingivalis are the most common pathogens that get attached to the surfaces of the tooth eliciting an immuneresponse from the host. Apart from this, contributing factors include genetics, environment and host factors[4].

In theadolescent population, aggressive periodontitis has a prevalence of below 1%and sometimes it may affect older patients. It affects both males and females.Some studies have suggested a predilection for female patients, particularly in the youngest age groups. The risk in black population is higher when compared to white population[5,6].

The main symptoms are pain and loosening of teeth in the affected areas. Gum bleeding may occur spontaneously or upon stimulation. Aggressive disease is diagnosed by inspection of the soft gum tissues around the teeth with a probe (clinical examination), by evaluation of the patient's x-ray films (radiographic examination) to determine the amount of bone loss around the teeth and by detecting the presence of pathogenic bacteria in the saliva (microbiological examination)[7].

The most common and conventional treatment for Aggressive periodontitis is Scaling and Root planingunder the coverage of systemic antibiotics. Among all

the antibiotics, the most efficacious in treating aggressive periodontitis is doxycycline. But, the overuse and misuse of antibiotics over time has led to the emergence of drug resistant microorganisms.

The use of antibiotics may also disturb the indigenous microflora of the body which includes Lactobacilli in the oral cavity. Hence, it is better to avoid the use of antibiotics that are highly active against Lactobacilli. Recently the trend has shifted towards the usage of probiotics and prebiotics.[8]

Probiotics are live micro-organisms with beneficial effects whereas Prebiotics are non digestible food components that stimulate probiotics. Synbiotic is a mixture of pre & probiotics and commonly includes Streptococcus faecalis, Clostridium butyricum, Bacillus mesentricus and Lactobacillus sporogenes. Here, the bacteria which are beneficial to health are stimulated to grow. They also act against those bacteria that are harmful and modify the immunity of oral mucosa.[9]

In the care of oral cavity, the use of probiotics is a breakthrough. Though there are many studies showing the benefits of probiotics in oral diseases, data among South Indian population are limited. Therefore this study has been undertaken to evaluate safety and efficacy of synbiotic in patients with aggressive periodontitis in our population.

REVIEW OF LITERATURE

PERIODONTITIS

Periodontal diseases comprise two inflammatory conditions-

1) Gingivitis and 2)Periodontitis. Both conditions affect tissues encircling and enclosing the tooth. The process is initiated with inflammation of the gum (gingivitis) and if it is not treated, slowly the inflammatory process advances to involve periodontal fibers and alveolar bone surrounding the tooth (periodontitis).[10]

Periodontitis is defined as "an inflammatory disease of the supporting tissues of the teeth caused by specific microorganisms or groups of specific microorganisms, resulting in progressive destruction of the periodontal ligament and alveolar bone with increased probing depth formation and clinical attachment loss".[11]

CLASSIFICATION OF PERIODONTITIS :[11]

1. Chronic Periodontitis
2. Aggressive Periodontitis
3. Periodontitis as a Manifestation of Systemic Diseases

CHRONIC PERIODONTITIS:

- Age of onset : adults > 35 years old
- Rate of disease progression : slow to moderate
- Presence of abundant plaque and calculus
- Different bacterial pattern
- Sub classified into localized (<30% of sites involved) and generalized (>30% of sites involved) forms.

AGGRESSIVE PERIODONTITIS:

When compared to chronic periodontitis aggressive periodontitis has the following main features

- Excepting for the disease process in periodontal region , they are otherwise healthy
- The loss of attachment of supporting connective tissues and destruction of bone are rapid.
- Positive family history
- Defect in the Neutrophils (PMNs) leading to failure in its functions of chemotaxis and phagocytosis.

The other presentations include

- The amount of microbial deposits does not coincide with the severity of the disease
- The organisms implicated are*Aggregatibacteractinomycetemcomitans* and in some cases *Porphyromonas gingivalis*
- Levels of prostaglandin E_2 (PGE_2) and interleukin 1β (IL) are increased
- Phagocytic abnormalities

According to the 1999 Consensus Report published by the American Academy of Periodontology (AAP), aggressive periodontitis was sub classified into localized and generalized forms.

Localized aggressive periodontitis (LAP):

- ✓ The onset is commonly at the time of puberty
- ✓ First molar/incisor are mostly involved
- ✓ Strong antibody response to the offending agent
- ✓ Inflammation of gingiva, edema, bleeding, pocketing

Generalized aggressive periodontitis (GAP):

- ✓ Less than 30 years age group are commonly involved
- ✓ Poor antibody response to agents causing infection

- ✓ The destruction of periodontal tissues are more pronounced and are episodic in nature
- ✓ It affects at least three permanent teeth other than first molars and incisors.
- ✓ When compared to LAP the destruction of bone is larger and is more rapid
- ✓ Bleeding, deep pocketing , periodontal abscess - present

PERIODONTITIS AS A MANIFESTATION OF SYSTEMIC DISEASES:

1. Hematologic disorders
    - ✓ Acquired neutropenia
    - ✓ Leukemias and Others
2. Genetic disorders
    - ✓ Familial and cyclic neutropenia
    - ✓ Down syndrome
    - ✓ Leukocyte adhesion deficiency syndromes
    - ✓ Glycogen storage disease
    - ✓ Infantile genetic agranulocytosis and Others
3. Not otherwise specified

EPIDEMIOLOGY:[12,13]

The prevalence of localized aggressive periodontitis is estimated to be below 1% in geographically diverse adolescent populations. It affects both the

sexes. Mostly the incidence is between puberty and twenty years of age. Larger incidence particularly in young female patient have been shown in some studies.

In the US national survey of adolescents aged 14 to 17, 0.53% had localized formand 0.13% had generalized form of the disease. In blacks, male teenagers had 2.9 times higher incidence than female adolescents, whereasin whites, females were at greater risk for the disease than males.

In a study of untreated periodontal disease conducted in Sri Lanka by Loe et al, 8% of the population had generalized aggressive periodontitis. In addition, blacks were at greater risk than whites for all forms of aggressive periodontitis.

ETIOLOGY AND PATHOGENESIS:[11]

Aggressive periodontitis has the feature of loosening of teeth and destruction of supporting tissues in the periodontal region at an early age. The aggressive nature is attributed to the expression of highly virulent causative agents or high levels of patient's susceptibility or both.

Microbiologic Factors:

The etiology of periodontitis is very complex including the oral biofilm which triggers the immuno-inflammatory response in a susceptible host. This destroys the periodontal region. Pathogenic bacteria are the primary etiology agents in the pathogenesis of periodontitis. There are about 700 different organisms in the

oral flora. Of these species, only some micro-organisms have been specifically associated with periodontal diseases.

The majority of periodontal pathogens are Gram-negative and strict anaerobe. Among the most important species, Aggregatibacter actinomycetemcomitans is the main cause of Aggressive form of the disease. Other bacteria are Porphyromonasgingivalis, Tannerella forsythia, Treponemadenticola, Fusobacteriumnucleatum, Prevotellaintermedia, Prevotellanigrescens, Campylobacter rectus, Eikenellacorrodens and Parvimonasmicra.Eventhough all these bacterial species have been associated with progression of periodontal destruction, A. actinomycetemcomitans is identified as primary organism causing this disease. This is based on the facts put forward by Tonetti and Mombelli which includes:

- A.actinomycetemcomitans is isolated in around 90% of the lesions of aggressive periodontitis
- Most of the patients who show clinical evidence of this disease have their serum showing higher levels of antibody titre to this organism. Studies have shown that there is a decrease in the bacterial load when the patient is successfully responding to treatment.
- A lot of virulence factors contributing to the disease have been attributed to this organism.

In some studies, no significant association was found between the presence of aggressive disease and A. actinomycetemcomitans. In other studies,increased levels of P. gingivalis, P. intermedia, Fusobacteriumnucleatum, C. rectus and Treponemadenticolawere found in patients with either localized or generalized form of the disease.

In addition, A.actinomycetemcomitans can be detected in healthy personswho are not suffering from any periodontal disease. This suggests that this organism is a part of the normal flora.

Immunologic Factors:

Studies were conducted to establish a link between immune regulators like the human leukocyte antigens (HLA) and aggressive periodontitis. Though the results were inconsistent, HLA-A9 and B15 have shown strong association with this disease.

Several researchers have shown that polymorphonuclear leukocytes (PMNs) are defective in patients with aggressive form. This can ultimately lead to failure of chemotaxis and phagocytosis.

In recent studies, a hyperresponsiveness of monocytes from localized aggressive periodontitis patients linked with their production of PGE 2 in response to lipopolysaccharide (LPS) have also been demonstrated. This hyperresponsive phenotype could lead to increased connective tissue or bone loss due to these excessive factors leading to destruction.

In addition, poorly functional inherited forms of monocyte FcyR II, the receptor for human IgG2 antibodies are found in increased levels in patients having localized aggressive periodontitis. The defective functions of PMN and monocyte may either be due to infection or may be of genetic origin.

Autoimmunity may also play a role in generalized aggressive periodontitis according to Anusaksathien and Dolby who found host antibodies to collagen, DNA and immunoglobulin G (IgG). Possible immune mechanisms include an increase in the expression of type II major histocompatibility complex (MHC) molecules, HLA DR43, altered helper or suppressor T-cell function, polyclonal activation of B cells by microbial plaque and genetic predisposition.

Genetic Factors:

Evidence suggests inherited immunological defects may be associated with this disease. For example, Van Dyke et al have shown that the abnormalities in neutrophils occurring in localized aggressive periodontitis is associated with familial clustering. This clustering suggests that the defects may be inherited.

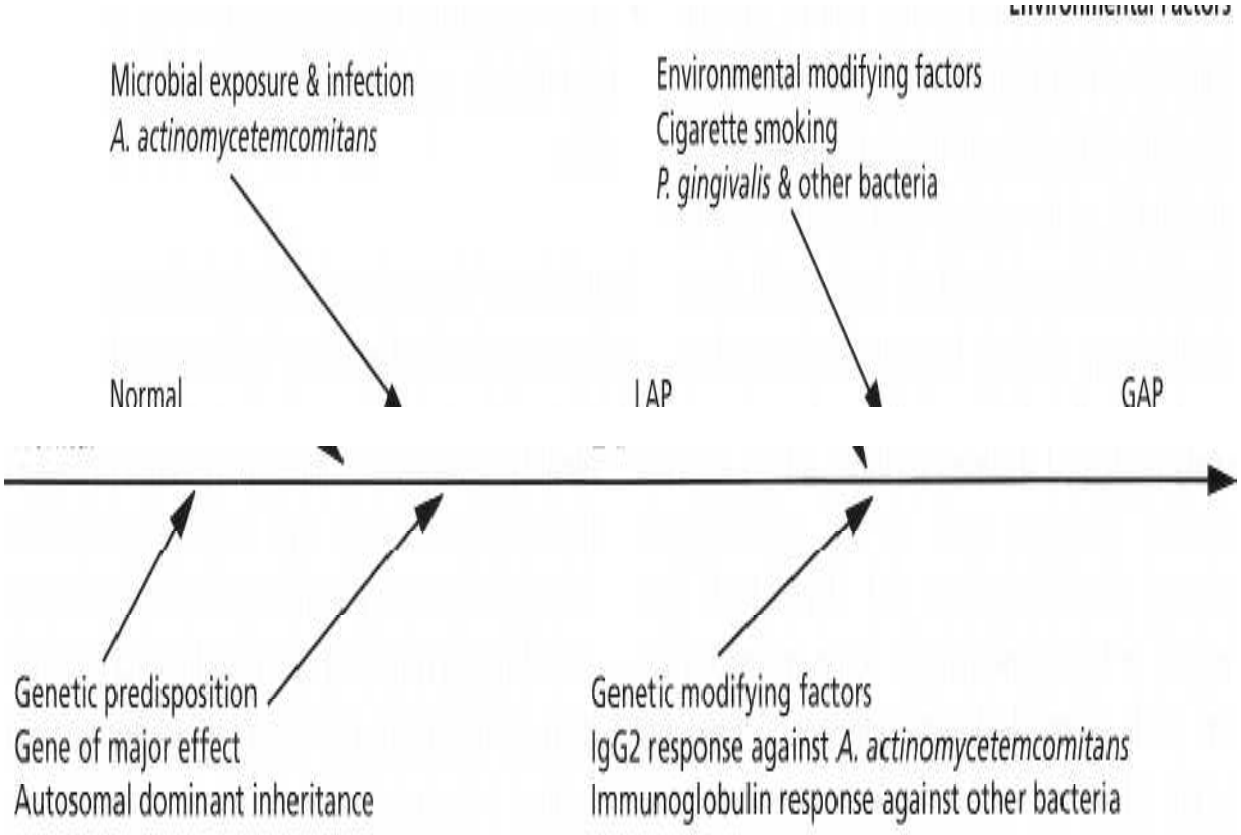
The main pathogen A. actinomycetemcomitans is genetically influenced.The antibody response against this pathogen is genetically controlled. Tonetti and Mombelli have summarized that the genes responsible for pathogenecity may be different in different ethnic groups explaining true genetic heterogeneity.

Environmental Factors:

Smoking is a risk factor for this disease. The severity of the disease particularly in young individuals depends on the amount and duration for which the individual smokes.Patients who smoke have more severe generalized aggressive periodontitis than non smokers who are suffering from the same disease. However, this is not the same in case of localized aggressive periodontitis.

Current concepts:

- The cause for aggressive periodontitis is multifactorial. Numerous factors interact in a complex manner resulting in the disease process.
- Inheritance of Aggressive periodontitis susceptibility is probably not sufficient.
- The environment also plays a role in the disease process. Exposure and the virulence of the organism plays a part.
- Host inability to effectively deal with the bacterial aggression and to avoid inflammatory tissue damage results in the initiation of the disease process.



It represents the interactions between environmental (e.g. cigarette smoking) and genetically controlled (e.g. IgG2 response to A.*actinomycetemcomitans*.) modifying factors leading to development of Localized (LAP) and Generalized Aggressive Periodontitis (GAP).

Clinical features:

- ✓ Clinically, the localized form does not involve more than two teeth and first molars/incisors are commonly affected, whereasin generalized form, at least three permanent teeth are involved other than first molars and incisors.

- ✓ Presence of periodontal pockets and the characteristic absence of inflammation are the main features of localized aggressive periodontitis.
- ✓ In most cases the plaques present on the diseased teeth is lesser when compared to the amount of destruction of periodontal tissues.
- ✓ A biofilm is formed over the diseased teeth by the plaque and this can in a rare instance lead to formation of calculus.
- ✓ When compared to chronic periodontitis , the progression of the disease is rapid and is about three to four times faster.[14]

Other clinical features:[15]

- Disto labial migration of the maxillary incisors
- Increasing teeth mobility
- The supporting structures get irritated during the act of mastication and this can lead on to a deep pain which is dull and radiating.
- Increased sensitivity to the stimulus of heat and tactile sensation on the denuded root surfaces can be present.

Radiographic features:

- Localized form of the disease shows characteristic arc-shaped radiolucent mirror image in the first molars starting from the distal aspect of second premolars to the mesial aspect of the second molar.

- The generalized form of the disease shows bony destruction in a generalized manner on radiographic images. The severity determines the amount of destruction. It may range from mild destruction of crestal bone to greater destruction of alveolar bone. Bony defects may be horizontal or vertical or both[16].

Diagnosis:

Early diagnosis plays a huge role in preventing the loss of attachment of periodontal soft tissues and loss of bone. The American Academy of periodontology has set some criteria for diagnosing. This includes history, clinical features and radiographic features with microbial examination if required.

History of loss of tooth at an earlier age in the family may be positive. In contrast to chronic periodontitis the amount of plaque and the amount of organisms deposited may not be consistent with the amount of destruction. Radiographs are taken in a serial manner and compared to establish the rate of destruction, thereby aiding in diagnosis.

Differential Diagnosis:

- Rapid nature of progression of the disease, age of onset, gingival microbiology, immune system alterations and a positive family history helps in differentiating aggressive forms from chronic forms of the disease.
- Systemic forms may be of hematologic or genetic origin can be differentiated by careful systemic examination, hematological and immunological assays.

Management:

The most common and conventional treatment approach ofAggressive Periodontitisis the mechanical therapyin the form of **Scaling and Root planing** (SRP) which is the process of removing the etiologic agents-dental plaque and calculus from the exposed surfaces of teeth andtheir root surfaces with periodontal scalers and periodontal curettes under local anaesthesia. In patients with severe defects, surgical therapy with graft placement is indicated.

Since the major periodontal pathogens may escape from the mechanical therapy, this conventional periodontal therapy (SRP) needs to be combined with systemic antibiotics.

Among all the antibiotics, doxycycline has been found to be the most efficacious in the treatment of aggressive periodontitis[17].

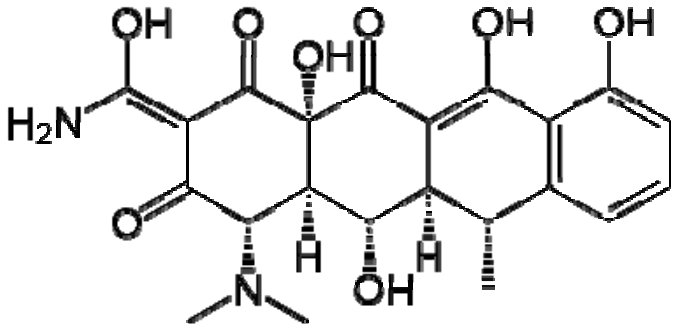
Doxycycline:[18,19]

Doxycycline is a broad-spectrum antibiotic of the tetracycline class.

Chemical name is (4S,4aR,5S,5aR,6R,12aS)-4-(dimethylamino)- 3,5,10,12,12a-pentahydroxy- 6-methyl- 1,11-dioxo- 1,4,4a,5,5a,6,11,12a-octahydrotetracene- 2-carboxamide

The molecular weight is 444.43 g / mol

Its molecular formula is $C_{22}H_{24}N_2O_8$ and it has the following structural formula.



DOXYCYCLINE

Pharmacological properties:

It inhibits protein synthesis by binding to 30S ribosomes in susceptible organisms.

Pharmacokinetic parameters:

- Bioavailability –95 to100%
- Plasma protein binding – 90%
- Metabolism – hepatic
- Half life – 18 to 24 hours
- Excretion – biliary

Dosage forms:

- Available as tablet, capsule, syrup and injectable powder forms.
- Oral dose is 200 mg initially, then 100-200mg once daily.

Common indications:

- Venereal diseases
- Atypical pneumonia
- Cholera
- Brucellosis
- Plague
- Relapsing fever
- Rickettsial infections
- Anthrax
- Leptospirosis
- Acne vulgaris
- Periodontitis

Adverse effects:

- Epigastric pain, nausea, vomiting and diarrhea
- Phototoxicity

Drug interactions:

- Enzyme inducers such as phenytoin, carbamazepine – reduce its serum level
- It can increase the action of warfarin by inhibiting the production of vitamin K in intestines.

Role of Doxycycline in Periodontitis:[20]

- The growth of the main organism is inhibited by Doxycycline aided by its tendency to concentrate in periodontal region.

- Additional features of anti-collagenase effect and it's regenerating capacity in bone plays a part in the cure.

Why new drug is needed?

Mechanical removal of plaque and calculus may not eliminate the periodontal pathogens completely from the root surfaces of teeth which necessitates the adjunctive therapy of systemic antibiotics. But, the misuse, overuse of antibiotics have led to drug resistance.[21]

Hence, time has come for the application of health-promoting bacteria (Synbiotics) in the management of aggressive periodontitis. They act mainly by preventing the growth of pathogenic bacteria and promoting the beneficial bacteria, thereby improving the periodontal health.[22]

SYNBIOTICS

Synbiotics are synergistic combination of probiotics and prebiotics. They impede the disease progression, modify plaque, alter the colonization of anaerobic bacteria, heal formed pockets and reduce loss of clinical attachment.

Prebiotics

Prebiotics are non-digestible food components that stimulate probiotics. Recently, the definition has been refined to include selectively fermented ingredients that allow specific changes, both in the composition and/or activity in the gastrointestinal microflora that confer benefits upon host well-being and health.[23]

Sources of prebiotics:[24]

- ✓ Soybeans
- ✓ Inulin sources
  - -Jerusalem artichoke
  - - Jicama
  - -Chicory root
- ✓ Raw oats
- ✓ Unrefined wheat
- ✓ Unrefined barley

- ✓ Mutated bacterial species of Clostridim butyricum, Streptococcus fecalis, Bacillus mesentricus

Commonly used prebiotics:[25,26]

1. Oligofructose
2. Inulin
3. Galacto oligosaccharides
4. Lactulose

Prebiotics with increased function have also been designed. They are derivatives of oligosaccharide which acts as receptors of epithelial cells. The pathogensits on these receptors rather than epithelial cells and thereby hinder their action.

Short-chain prebiotics:

e.g., oligofructose - act on right side of the colon providing nourishment to the bacteria in that area.

Longer chain prebiotics:

e.g., Inulin – act predominantly in the left side of the colon.

Full-spectrum prebiotics:

e.g., oligofructose-enriched inulin (OEI) - act throughout the colon.

These full-spectrum prebiotics are commonly used in periodontal healthcare.

MECHANISM OF ACTION OF PREBIOTICS:[27,28]

- Helps in the growth of beneficial microflora
- Cytokines like IL-10 and interferon γ are produced in larger quantities
- Secretion of IgA is enhanced
- Immune response to the infective organism is modified.
- The barrier in the gut mucosa is modified.

Probiotics:

Live microorganisms that are provided to our body to produce beneficial effects are called as probiotics. The concept of probiotics was first introduced by Elie Metchnikoff, a Russian scientist, following his observation that Bulgarian people had a longer life span due to the consumption of fermented milk containing viable bacteria.

The term "Probiotic" was initially proposed by Lilley and Stillwell in 1965. The word "Probiotics" was originally derived from a composite of the Latin preposition *pro* ("for") and the Greek noun bios ("life")[29]. First probiotic species to be introduced in research was *Lactobacillus acidophilus* by Hull et al in 1984 followed by *Bifidobacteriumbifidum*by Holcombh et al in 1991[30].Probiotics organisms have been classified by FDA as Generally Regarded as Safe (GRAS).

Common sources of probiotics:[31]

- Kombucha - a fermented tea
- Kefir
- Ginger Beer
- Moroccan preserved lemons
- Sour pickles
- Dairy products

Criteria of an ideal microorganism to be used as probiotics:[32,33,34]

- ❖ Should be of human origin
- ❖ Should be non pathogenic
- ❖ High cell viability, resistant to low pH and acids
- ❖ Persisting nature
- ❖ Capacity to adhere
- ❖ Signal sending capacity to cells involved in immune response.
- ❖ Resistance to processing
- ❖ Ability to regulate local metabolism.

Essential requisites for microorganisms to exert probiotic properties in the oral cavity:

- ❖ To adhere to the saliva coated surfaces
- ❖ To colonize and grow in the mouth

- ❖ To inhibit oral pathogens
- ❖ To resist the oral environmental conditions and defense mechanisms
- ❖ To be also safe for the host.

The mechanisms by which beneficial bacteria act are:[35]

- o By passively occupying a niche that may be otherwise colonized by pathogens
- o Actively limiting the pathogens ability to adhere to appropriate surfaces
- o By adversely affecting the growth or vitality of the pathogen
- o By affecting the ability of the pathogen to produce virulence factors
- o By degrading the virulence factors produced by the pathogen
- o By providing bioactive or regulatory peptides
- o Through modifying immune system, altering permeability of epithelial cells and translocation.

Mechanisms of action of Probiotics in periodontal diseases:

- ❖ **Inhibition of specific organisms**
    - Invasion by microorganism and formation of biofilm are inhibited.
    - By producing substances like hydrogen peroxide and bacteriocins that kill the microorganisms.

❖ **Effects on host response**

- Inhibition of collagenases and reduction of inflammation associated molecules
- Induction of expression of cytoprotective proteins on host cell surfaces
- Prevention of cytokine-induced apoptosis
- Modulation of pro-inflammatory pathways induced by pathogens
- Modifying the response of immune system of host.

IMPORTANT STRAINS IN PROBIOTIC FORMULATIONS:[36]

1. Lactobacillus.
2. Streptococcus
3. Bacillus species
4. Clostridium
5. Bifidobacteria
6. Streptomyces
7. Yeasts and moulds like Saccharomyces boulardii

Lactobacillus species:

They are gram positive, lactic acid producing bacteria. They are found mainly in the small intestine. Lactobacillus sporogenes is non pathogenic bacterium

naturally occurring in intestine. It is responsible for synthesis of vitamin B complex and also responsible for synthesis of digestive enzymes. These spores proliferate in the small intestine and produce lactic acid which inhibits the enteric pathogenic organisms.

Lactobacillus rhamnosus improves body immunity and prevents antibiotic associated diarrhoea. Lactobacillus reuteri is used in prevention of H.pylori and gingival infection. Lactobacillus acidophilus and Lactobacillus plantarum are also used as probiotic supplements.

Oral administration of tablets containing L.salivariussignificantly lowered the numbers of periodontal pathogenic bacteria in plaque and improved periodontal health in two double blinded, placebo controlled, randomized clinical trials (Shimauchi *et al*, 2008 and Mayanagi *et al*, 2009)[37,38].

Lactobacillus reuteriimproved the gingival bleeding index and reduced bacterial plaque (Krasse *et al*, 2006)[39] and caused a decline in gingival bleeding and inflammatory mediators (Twetman *et al*,2009)[40]**.** The use of probiotic lozenges containing Lactobacillus brevis was recently reported to significantly improve the periodontal health of subjects with aggressive periodontitis (Shah *et al*. 2013).

Bifidobacterium species:

Bifidobacteria are gram positive, micro aerophillic that are highly prevalent in human intestines. Bifidobacterium animalis and Bifidobacterium longum are used in infantile diarrhea.

In a double-blinded, randomized crossover trial, Caglar et alfound that salivary S.mutanscounts significantly declined after consumption of ice-cream containing Bifido bacterium lactis (Caglar et al, 2008).[41]

Lee et al. reported that Bifidobacterium adolescentisand Bifidobacterium longum inhibited both Streptococcus mutansand Streptococcus sobrinus, both reported as being cariogenic (Lee et al, 2011).[42]

Streptococcus faecalis:

They are gram positive, aerobic, non spore forming cocci that proliferate with bacillus mesentricusand clostridium butyricum to produce lactic acid which inhibit harmful bacteria including periodontal pathogens.

Clostridium butyricum:

They are live gram positive spore forming bacilli producing butyric acid and acetic acid which decrease intestinal pH and prevents growth of harmful bacteria.

Bacillus species:

Bacillus mesentricus and spores of Bacillus clausii have probiotic action. Live gram positive spore forming bacilli that produces an amylolytic enzyme and protease to activate proliferation of streptococcus[43].

USES OF PROBIOTICS:

GASTROINTESTINAL TRACT:

Probiotics containing β galactosidase helps in improving lactose intolerance. Intake of probiotics like Saccharomyces cerevisiae helps in degradation of sucrose in children with sucrase deficiency. Deficiency of beneficial microorganisms and overgrowth of clostridium difficle are responsible for the occurrence of Antibiotic associated diarrhea(Hawrelak et al 2005).[44] Use of Saccharomyces boulardii has been shown to improve the condition by replacing the beneficial micro flora.

Probiotics are also being used in prevention and treatment of Rotavirus associated diarrhoea. The effects are due to production of acids, hydrogen peroxide, antimicrobial substances, competition for nutrients or adhesion receptors, antitoxin actions and stimulation of immune system. Probiotics have also been found to be effective in antibiotic associated diarrhoea. Lactobacillus reduces the risk of colorectal cancer by reducing the activity of certain fecal enzymes which convert the procarcinogens to carcinogens (Ouwehand et al. 2002)[45].

Eradication of Helicobacter pylori

Several lactobacilli and bifidobacterial strains as well as Bacillus clausiiappear to reduce the side effects of antibiotic therapies and improve patient compliance. Several strains were effective in decreasing side effects and increasing the eradication rates (Gaon et al2002)[46].

Hepatic encephalopathy

It is a complication of liver cirrhosis that can be prevented and treated by lactulose which is a prebiotic.

Irritable bowel syndrome (IBS)

Compared to placebo,probiotics are found to have beneficial effects. Studies have shown that it reduces bloating and flatulence of abdomen. Several studies have demonstrated significant therapeutic gains with probiotics in comparison with placebo. Some strains may ameliorate pain and provide global relief (B. infantis35624) in addition. Lactobacillus reuteri improves colicky symptoms within one week.

HALITOSIS:

Probiotics such as Streptococcus salivarius produces bacteriocins which inhibit bacteria producing Volatile sulphur compounds (VSC) that are responsible for halitosis and hence used in the treatment and prevention of halitosis[47].

DENTAL CARIES:

Probiotics prevent the cariogenic bacteria from adhering and therefore reduce their chances of producing acidic byproducts that are responsible for the development of dental caries.[48]

PERIODONTAL DISEASES:

By preventing the colonization of periodonto pathogens, probiotics exhibit lower probing depths and less loss of clinical attachment and improve both clinical and microbiologic parameters in gingivitis and periodontitis patients.

ATOPIC DISEASE:

Lactobacilli reduce the gut permeability, increases gut specific IgA response, promotes the barrier function of the intestines by restoring beneficial microbes to normal level. They also enhance the production of TGF-β and IL-10 and increase the level of cytokines that promote the production of IgE antibodies.[49]

UROGENITAL INFECTIONS:

L.rhamnosus and L.reuteri strains when applied topically helps in prevention of urogenital infections.[50]

ENT INFECTIONS

α-Hemolytic Streptococci have an interfering activity against pathogens that cause otitis media.[50]

APHTHOUS ULCER

Probiotics are beneficial in treatment of recurrent aphthous ulcers of the mouth. The ability of lactobacillus to increase the activity of phagocytes must be the key factor in combating recurrent aphthous ulcer[50].

EFFECT ON CANDIDIAL INFECTIONS

Probiotics reduce the prevalence of oral candidiasis and risk of hypo salivation in elderly.[51]

Suitable formulation of Probiotics for periodontal disease management:

Lozenges, Chewing gums, Tooth pastes and Mouth rinse - these things facilitate enough contact with oral cavity and also help in the adhesion of probiotics.[52,53]

Adverse effects:

Probiotics are generally considered safe and well tolerated with bloating and flatulence occurring frequently[54].

RATIONALE OF USING SYNBIOTICS IN AGGRESSIVE PERIODONTITIS:

The main pathogenic agents associated with aggressive periodontitis areAggregatibacter actinomycetemcomitans, P. gingivalis, Treponema denticolaand Tannerella forsythus. These bacteria have a variety of virulent characteristics allowing them to colonize the subgingival sites, escape the host's defense system and cause tissue damage. The persistence of the host's immune response also constitutes a determining factor in progression of the disease.

The beneficial effects of probiotics in periodontal diseases have been explored in many studies. The common mechanism of action includes inhibition of pathogen adhesion, colonization through the production of antimicrobial substances and modulation of host immune response.

Many studies have shown that probiotics can reduce the probing depths and clinical attachment loss and significantly improve the periodontal health of subjects with aggressive periodontitis.

Hence, this approach may provide a valuable addition or alternative to the treatment options for periodontitis.

OBJECTIVE

OBJECTIVE:

To evaluate the efficacy and safety of Synbiotic as an Add on therapy to Standard treatment in the management of patients with Aggressive Periodontitis compared to Standard treatment alone.

PRIMARY END POINT:

- Reduction in Probing depth & Clinical attachment loss

SECONDARY END POINT:

- Reduction in Oral hygiene index & Gingival bleeding index

METHODOLOGY

The study was conducted in patients diagnosed with Aggressive Periodontitis and attending outpatient department of Periodontics, GovernmentDental College, Chennai.

Study design:

A Randomized, Prospective, Placebo controlled, Interventional study.

Study population:

Patients (18-30yrs old) with Aggressive Periodontitis (localized & generalized) attending outpatient department of Periodontics

Study center:

Institute of Pharmacology, Madras Medical College in collaboration with Department of Periodontics, Government Dental College, Chennai.

Study period:

August 2014 to April 2015.

Study duration:

Treatment period of 8 weeks and Post treatment follow up period of 4 weeks per patient.

Sample size:

60 patients (Control group - 30,Study group - 30).

Inclusion criteria:

- Age: 18-30 years
- Sex-both genders
- Patients recently diagnosed with Aggressive Periodontitis
- Patients willing to give written informed consent and come for follow up
- Patients adhering to oral hygiene instructions and education.

Exclusion criteria:

- Smokers and Alcoholics
- Pregnant and lactating women
- Patients treated with pre / probiotics in the last one month
- Patients allergic to prebiotics/probiotics
- Participation in another clinical study in the last three months
- Patients with Diabetes,Hypertension, chronic systemic illness of liver, heart, Kidney and HIV infection/AIDS.

Study procedure:

The study was conducted after obtaining the approval from Institutional Ethics Committee, Madras Medical College and it was done in accordance with declaration of Helsinki and Good Clinical practice (GCP) guidelines.

Patients diagnosed with Aggressive Periodontitis attending the Outpatient department of Periodontics, Government Dental College and Hospital were explained about the study purpose, procedure and benefits of the study.

After obtaining writteninformed consent in patient's own language, the study was carried out. The demographic details of the patients were recorded.The subjects were screened by complete medical and dental history, clinical and oral examination and laboratory investigations. Those who fulfilled all the inclusion and exclusion criteria were enrolled in the study.

Randomization:

The enrolled patients were randomized by simple randomization into either control group or study group and received the respective therapy. Patients were blinded to the groups they were assigned to.

- Control group (n=30) –Standard therapy + Placebo
- Study group (n=30) – Standard therapy + Synbiotic

TREATMENT PLAN:

Standard treatment:

- Scaling and Root Planing (SRP)
- Cap. Doxycycline 100 mg twice (1-0-1) daily for one week

Control group:

Standard treatment

+

Placebo – one lozenge twice (1-0-1) daily for 8 weeks
(Lactose containing lozenge-identical in colour, size and shape to the study drug)

Study group:

Standard treatment

+

Synbiotic–one lozenge twice (1-0-1)daily for 8 weeks.

Composition of synbiotic:

Each lozenge contains

- Streptococcus faecalis T-110 JPC - 30 million
- Clostridium butyricum TO-A IHS - 2 million
- Bacillus mesentricus TO-A JPC - 1 million
- Lactobacillus sporogenes IHS - 50 million.

Patients were instructed to place the lozenges in the oral cavity for a few minutes, allowing them to dissolve to increase the contact time in the mouth where it acts locally.

ASSESSMENT PARAMETERS:

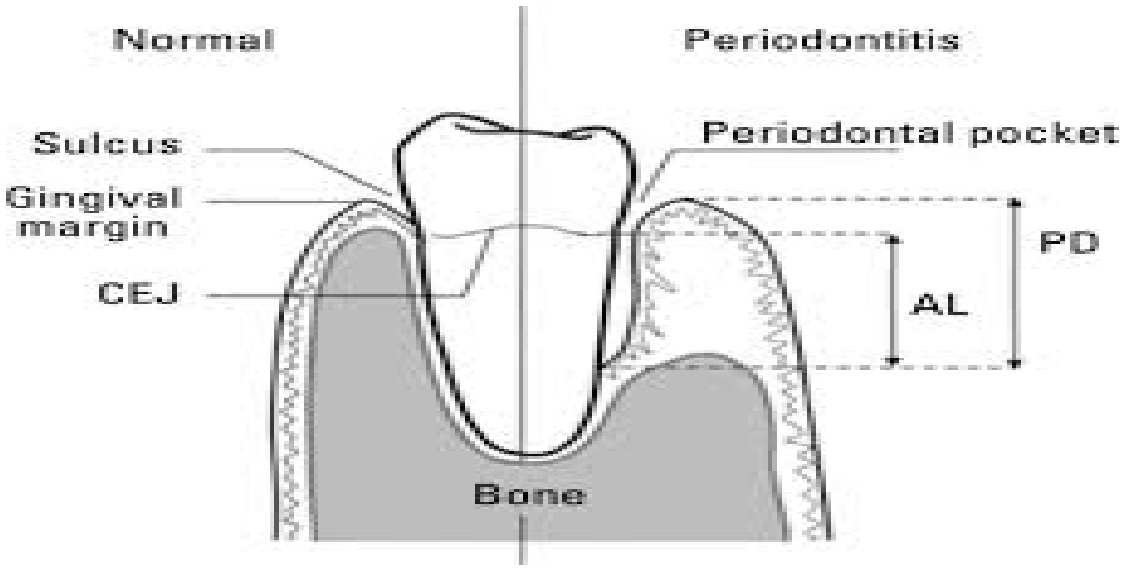
- Probing depth
- Clinical attachment loss
- Oral hygiene index
- Gingival bleeding index

Standardized tables are used to measure all these parameters. In these tables, two digit numbers are used. First digit denotes number of quadrant; second digit denotes number of tooth in that particular quadrant. Dentition has been divided into four quadrants; each quadrant has eight teeth.

Probing Depth (PD) & Clinical Attachment Loss (CAL) (in mm):[7,11,55]

PD – distance from the free end of the gingival margin to the bottom of the periodontal pocket

CAL – distance from Cemento Enamel Junction (CEJ) to the bottom of the periodontal pocket



WILLIAMS GRADUATED PERIODONTAL PROBE

- Markings include 1,2,3,5,7,8,9 and 10 mm with 4mm and 6mm missing.
- For ease in measuring .

This probe whose end is blunt, is thin and long. The main use of this probe is to assess the health of the periodontal region by means of measuring the depths of the pockets formed around the tooth. This also has markings which can be utilized for measuring accurately.

The tip of this instrument is placed with light pressure into the gingival sulcus, which is an area of potential space between a tooth and the surrounding tissue. It is important to keep the periodontal probe parallel to the contours of the root of the tooth and to insert the probe down to the base of the pocket. This results in obscuring a section of the periodontal probe's tip. The first marking visible above the pocket indicates the measurement of the pocket depth. It has been found that the average, healthy pocket depth is around 3 mm with no bleeding upon probing. Depths greater than 3 mm can be associated with attachment loss of the tooth to the surrounding alveolar bone, which is a characteristic sign found in periodontitis.

According to the American Academy of Periodontology, the classification of severity is as follows:

Mild: 1-2mm of attachment loss

Moderate: 3-4mm of attachment loss

Severe: ≥5mm of attachment loss

Maxillary:

Palatal

CAL																
PD																
	18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28
PD																
CAL																

Buccal

Mandibular:

Lingual

CAL																
PD																
	48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38
PD																
CAL																

Buccal

Oral Hygiene Index(OHI):[7,11]

OHI=Debris Index (DI) + Calculus Index (CI)

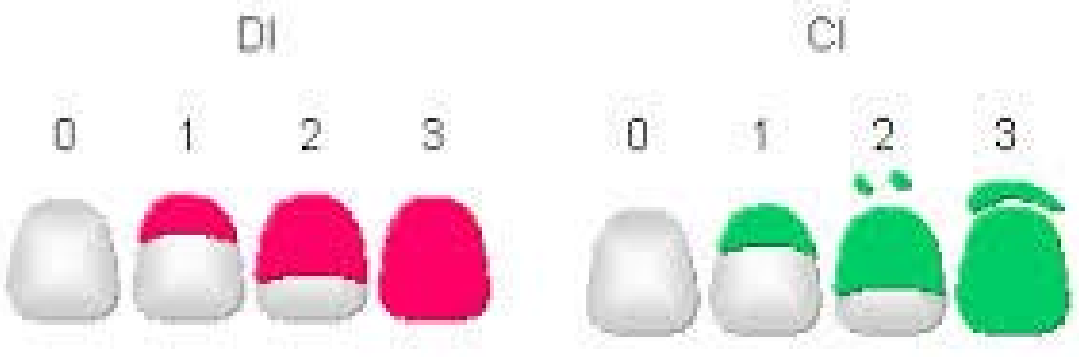
Debris or Calculus index=Total score/Number of teeth examined

Criteria for classifying debris:

Scores	Criteria
0	No debris or stain present
1	Soft debris covering not more than one third of the tooth surface, or presence of extrinsic stains without other debris regardless of surface area covered
2	Soft debris covering more than one third, but not more than two thirds, of the exposed tooth surface.
3	Soft debris covering more than two thirds of the exposed tooth surface.

Criteria for classifying calculus:

Scores	Criteria
0	No calculus present
1	Supragingival calculus covering not more than third of the exposed tooth surface.
2	Supragingival calculus covering more than one third but not more than two thirds of the exposed tooth surface or the presence of individual flecks of subgingival calculus around the cervical portion of the tooth or both.
3	Supragingival calculus covering more than two third of the exposed tooth surface or a continuos heavy band of subgingival calculus around the cervical portion of the tooth or both.



Debris index-score:

16	11	26
46	31	36

DI-S=

Calculus index-score:

16	11	26
46	31	36

CI-S=

INFERENCE:

0.0 –1.2 = Good oral hygiene

1.3 – 3.0 = Fair oral hygiene

3.1 – 6.0 = Poor oral hygiene

Gingival Bleeding Index:[7,11,56]

Bleeding on probing (BoP), even with a gentle touch can occur in periodontitis. It is due to the periodontal probe damaging the increased blood vessels in the capillary plexus of the lamina propria, which are close to the tooth surface because of the ulceration of the junctional epithelium (JE). The presence of bleeding is one of the first clinical signs of active periodontal disease.

18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28
48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38

If Bleeding on Probing (BoP) is present, it is recorded as +

If Bleeding on Probing (BoP) is absent , it is recorded as --

SCREENING:

- Written informed consent obtained.
- Demographic details obtained.
- Medical and Dental history taken and recorded.
- Vital signs recorded.
- General, Systemic & local (intra oral) examination done.
- Laboratory investigations done.
- X ray - Intra Oral Peri-apical radiograph / Orthopantamogram taken.

VISIT 1 (Baseline):

- Randomization done.
- Vital signs recorded.
- Oral hygiene index &Gingival bleeding index measured.
- Probing depth & Clinical attachment loss measured.
- Scaling and Root planing was done.
- Study drugs were issued for 2 weeks to respective groups.

- Instructed to return the empty bottles and strips during subsequent visit.
- Patients were instructed to report if any adverse events occur.

VISIT 2 (end of 2 weeks)

- Vital signs recorded.
- Patients were asked to return empty bottles to check compliance.
- Intra oral examination was done.
- Adverse events monitored.
- Study medication issued for subsequent 2 weeks.

VISIT 3 (end of 4 weeks)

- Vital signs recorded.
- Patients were asked to return empty bottles to check compliance.
- Intra oral examination was done.
- Adverse events monitored.
- Oral hygiene index &Gingival bleeding index measured.

- Probing depth & Clinical attachment lossmeasured.
- Study medication issued for subsequent 2 weeks.

VISIT 4 (end of 6 weeks)

- Vital signs recorded.
- Patients were asked to return empty bottles to check compliance.
- Intra oral examination was done.
- Adverse events monitored.
- Study medication issued for subsequent 2 weeks.

VISIT 5 (end of 8 weeks)

- Vital signs recorded.
- Patients were asked to return empty bottles to check compliance.
- Intra oral examination was done.
- Adverse events monitored.
- Oral hygiene index &Gingival bleeding index measured.

- Probing depth & Clinical attachment lossmeasured.
- Laboratory investigations done.

VISIT 6 (end of 12 weeks)

- Vital signs recorded.
- Intra oral examination was done.
- Oral hygiene index &Gingival bleeding indexmeasured.
- Probing depth & Clinical attachment lossmeasured.

Lab investigations:

The following laboratory investigationswere performed in the patients on screening and at the end of 8 weeks.

- Haematology - Haemoglobin, Total leucocyte count, Differential count.
- Blood glucose

Follow up:

The patients were further followed up for a post treatment period of 4 weeks for the assessment of aggressive periodontitis.

After the completion of 12 weeks of study period, the patients were provided appropriate dental care at Department of Periodontics, Government Dental College, Chennai.

Adverse events:

Any adverse event reported by the patient or observed by the dentist during the study was recorded. The onset of adverse event, causal relationship to the study drug and action taken was recorded. Appropriate medical care was provided.

Withdrawal:

During the study period the subject was allowed to withdraw his/her voluntary consent and opt out of study. Similarly at the discretion of the investigator, the subjects were withdrawn from the study if any serious adverse event was reported by the patient or observed by the dentist.

Statistical analysis:

The obtained data was analyzed statistically.

Distribution of age was analyzed using one way ANOVA and Sex distribution was analyzed by Pearson chi- square test.

The biochemical investigations were performed at baseline and at the end of 8 weeks. The differences within the groups before and after treatment were analyzed using student's paired t- test.

The difference within the groups in probing depth, clinical attachment loss and oral hygiene index score was analyzed using students paired t-test. Similarly the difference between the control and test groups was analyzed using independent t-test. Gingival bleeding index was analyzed by Pearson chi- square test.

Statistical analysis was done by using SPSS software version 21.

p value <0.05 was considered to be statistically significant.

RESULTS

This study was conducted to evaluate the efficacy and safety of synbiotic as add on therapy to Standard treatment in patients with Aggressive Periodontitis compared to standard treatment alone.

106 patients were screened, of which 38 patients were excluded from the study based on exclusion criteria and 8 patients who were eligible for the study were not willing to participate.

Thereby 60 patients were enrolled in this study and were randomized into either of the 2 groups: Control group [Standard therapy + Placebo] and Study group [Standard therapy +Synbiotic]. Each group consisted of 30 patients. All the enrolled patients completed the study.

STUDY FLOW CHART

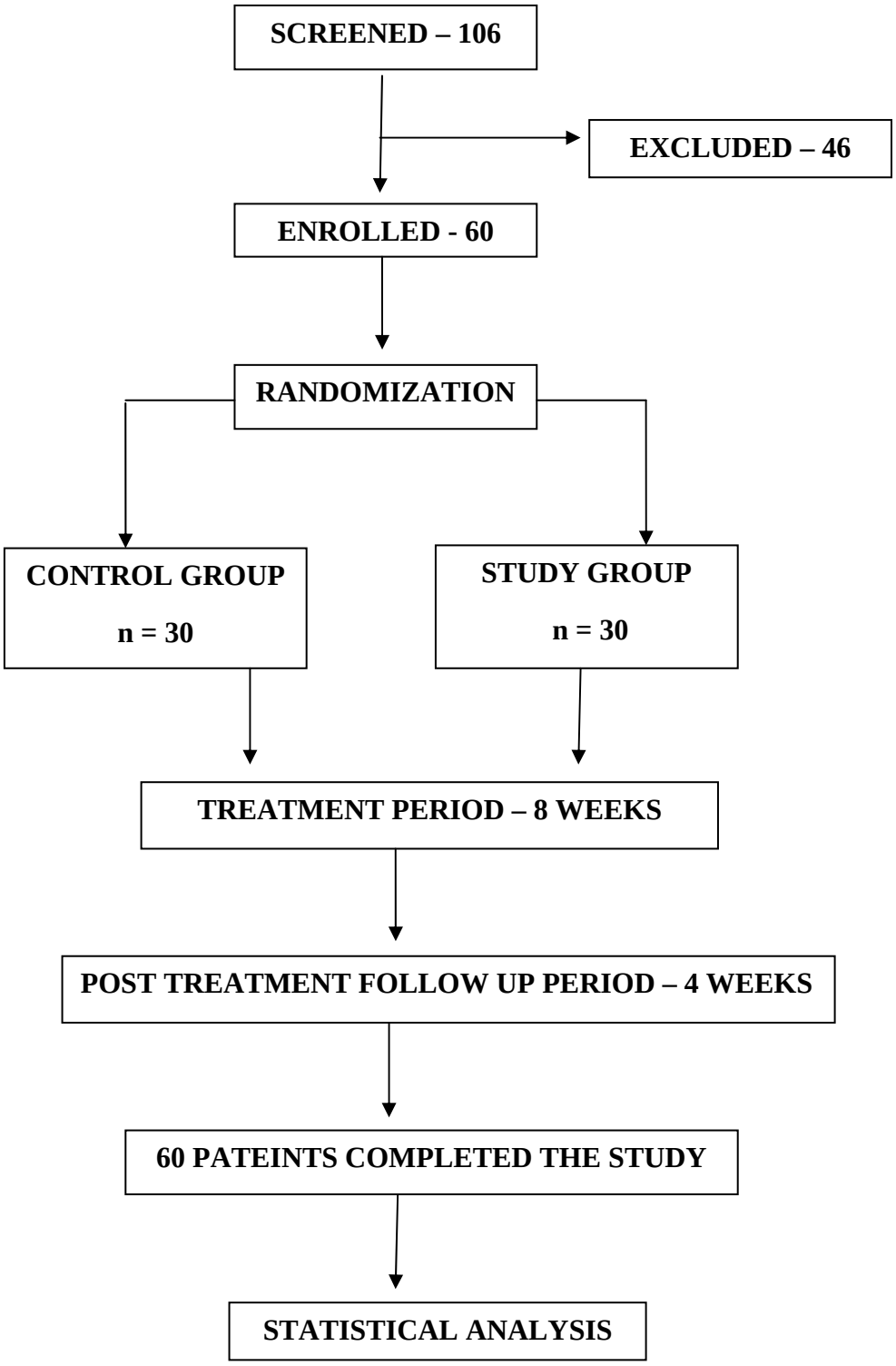


TABLE-1: MEAN AGE DISTRIBUTION

GROUP	NO. OF PATIENTS (n)	MEAN AGE IN YEARS	S.D
CONTROL	30	23.4	3.05
STUDY	30	23.5	3.49
p-VALUE	p = 0.907		

Table-1 shows the Mean Age Distribution of patients among the control and study groups. The mean age of patients in control group was 23.4 and in the study group was 23.5.There was no statistically significant difference in mean age between the groups.

FIGURE -1 MEAN AGE DISTRIBUTION

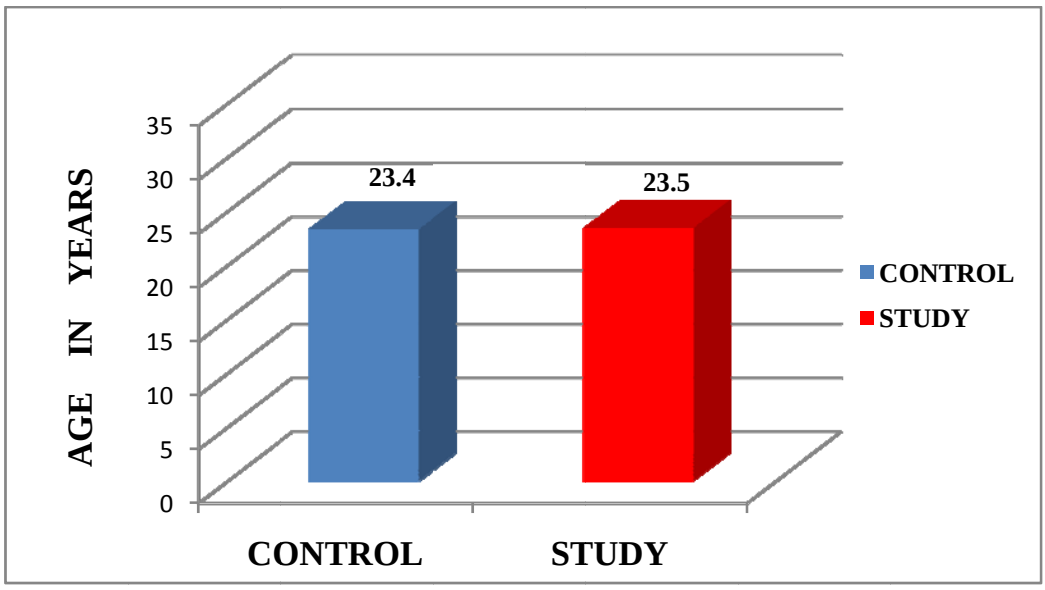


Figure-1 indicates the Mean Age Distribution of patients among control and study groups

TABLE-2: GENDER DISTRIBUTION

GROUP	MALE		FEMALE		TOTAL	
	N	%	N	%	N	%
CONTROL	13	43	17	57	30	100
STUDY	12	40	18	60	30	100

Table 2 shows the distribution of sex in the control and study groups.Percentage of females was higher than males. There was no statistically significant difference between the groups.

FIGURE-2: GENDER DISTRIBUTION

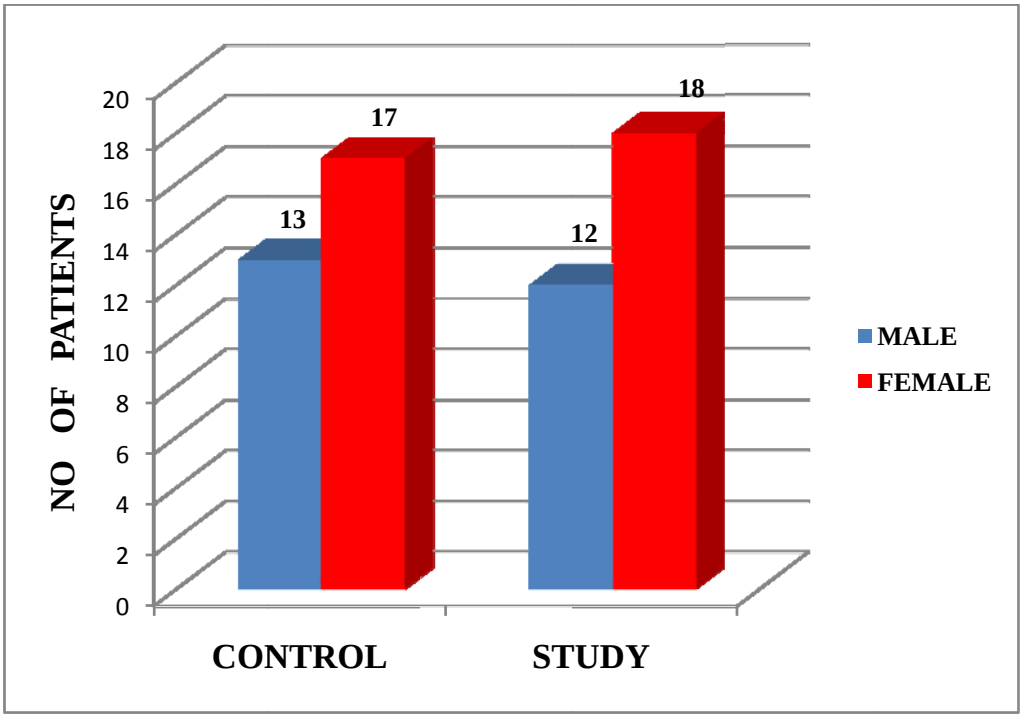


Figure-2 is the graphical representation of table-2

TABLE-3: TYPES OF AGGRESSIVE PERIODONTITIS

GROUP	LOCALIZED	GENERALIZED	TOTAL
CONTROL	10	20	30
STUDY	9	21	30
p-VALUE	p = 0.92		

Table-3 shows the types of aggressive periodontitis in both the control and study groups.Generalized aggressive periodontitis was more common in both the groups. There was no statistically significant difference between the groups.

FIGURE-3: TYPES OF AGGRESSIVE PERIODONTITIS

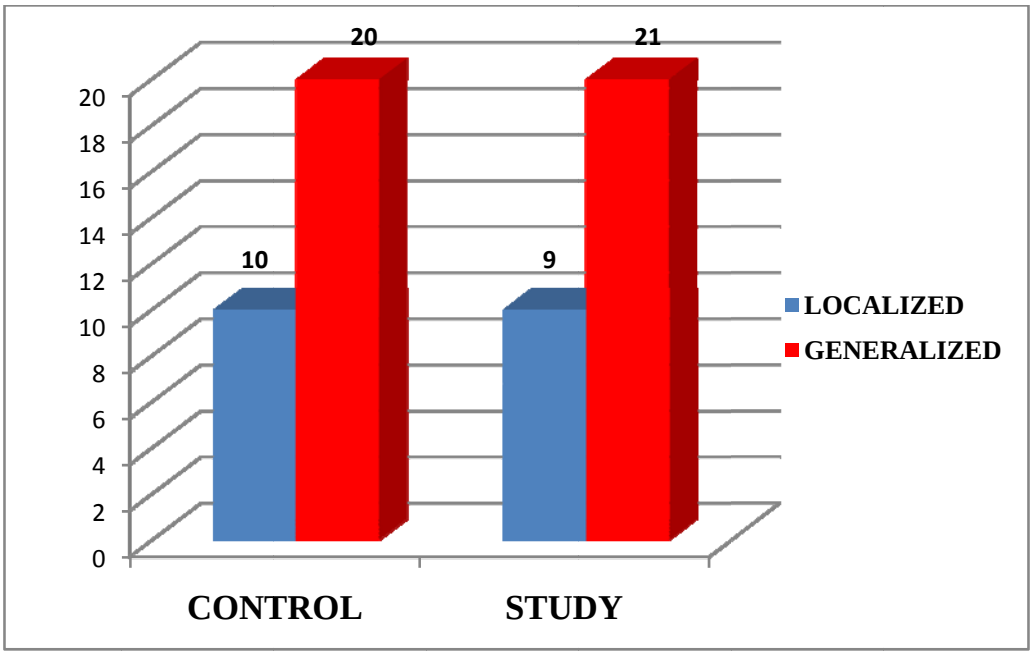


Figure-3 is the graphical representation of table-3

TABLE-4:MEAN PROBING DEPTH(in mm):

	CONTROL GROUP		STUDY GROUP		INDEPENDENT T-TEST
	MEAN	SD	MEAN	SD	
BASELINE	5.63	0.71	5.60	0.67	p=0.85
WEEK 4	4.46	0.73	3.66	0.75	P<0.01
WEEK 8	3.69	0.66	3.13	0.34	P<0.01
WEEK 12	3.60	0.49	3.10	0.30	P<0.01
p-VALUE	p<0.01		p<0.01		

Table-4 shows mean probing depth in both the groups from baseline to week 12.

- Statistical analysis within the group showed a significant reduction in mean probing depth in both the control and study groups.
- Comparison between the groups showed statistically significant reduction in mean probing depth from week 4 onwards.
- Post treatment follow up period at week 12 showed lesser mean probing depth in the study group than the control group.

FIGURE-4 MEAN PROBING DEPTH(in mm):

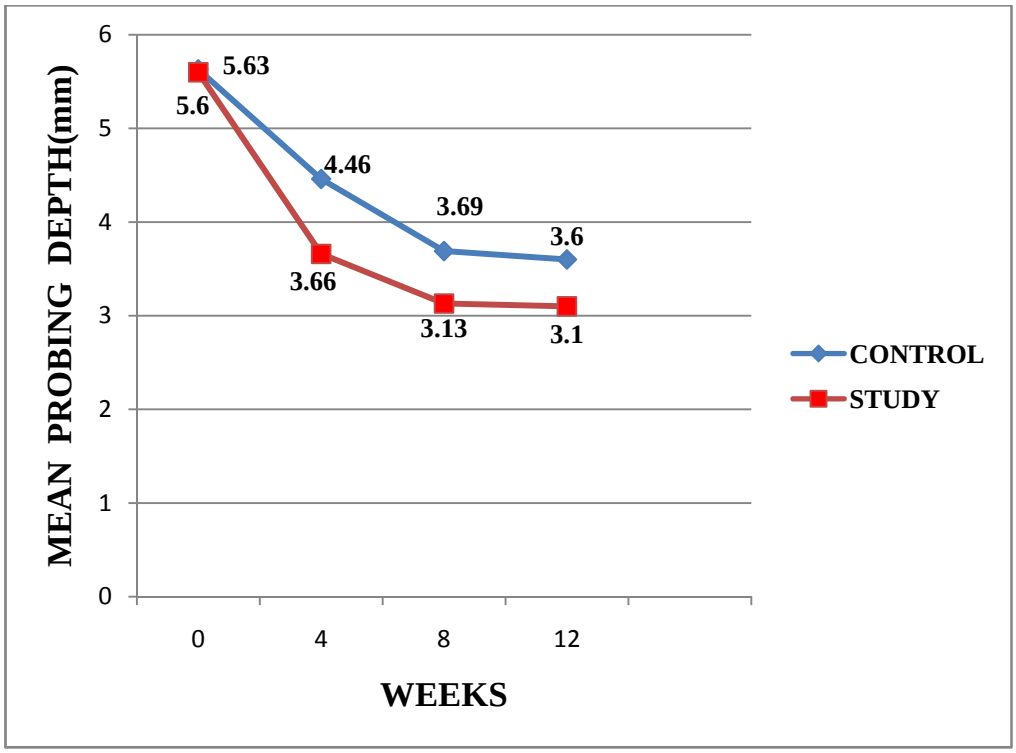


Figure-4 is the graphical representation of table-4.

TABLE-4A: MEAN PROBING DEPTH(in mm)

BASELINE vs 8 WEEKS

	CONTROL GROUP	STUDY GROUP	p-VALUE
BASELINE	**5.63**	**5.60**	**= 0.85**
8 WEEKS	**3.69**	**3.13**	**<0.01**

Table-4A shows mean probing depth in both the groups at baseline and week 8.

- There was a statistically significant reduction in mean probing depth in the study group at week 8 ($p < 0.01$).

FIGURE-4A: MEAN PROBING DEPTH(in mm)

BASELINE vs. 8 WEEKS

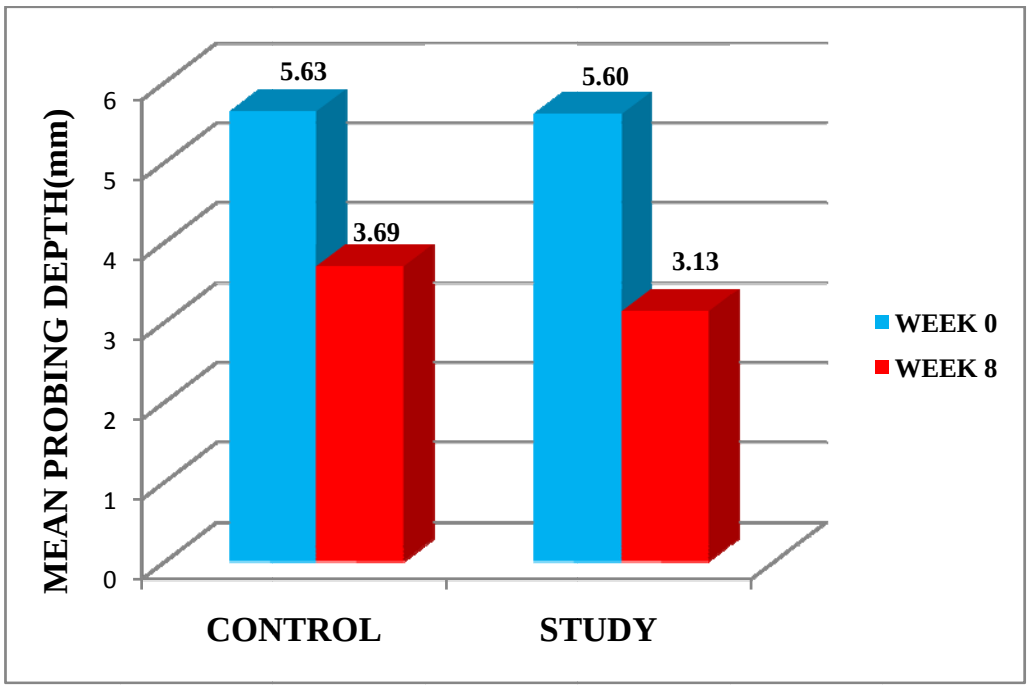


Figure-4A is the graphical representation of table-4A.

TABLE-5:MEAN CLINICAL ATTACHMENT LOSS(in mm):

	CONTROL GROUP		STUDY GROUP		INDEPENDENT T-TEST
	MEAN	SD	MEAN	SD	
BASELINE	7.36	0.76	7.46	0.77	p=0.62
WEEK 4	6.17	0.75	5.53	0.78	P=0.02
WEEK 8	5.37	0.72	5.00	0.53	P<0.01
WEEK 12	5.36	0.71	4.96	0.49	P<0.01
p-VALUE	p<0.01		p<0.01		

Table-5 shows mean clinical attachment loss in both groups from baseline to week 12.

- Statistical analysis within the group showed a significant reduction in mean clinical attachment loss in both the control and study groups.
- Comparison between the groups showed statistically significant reduction in mean clinical attachment loss from week 4 onwards.
- Post treatment follow up period at week 12 showed lesser mean clinical attachment loss in the study group than the control group.

FIGURE-5 MEAN CLINICAL ATTACHMENT LOSS(in mm):

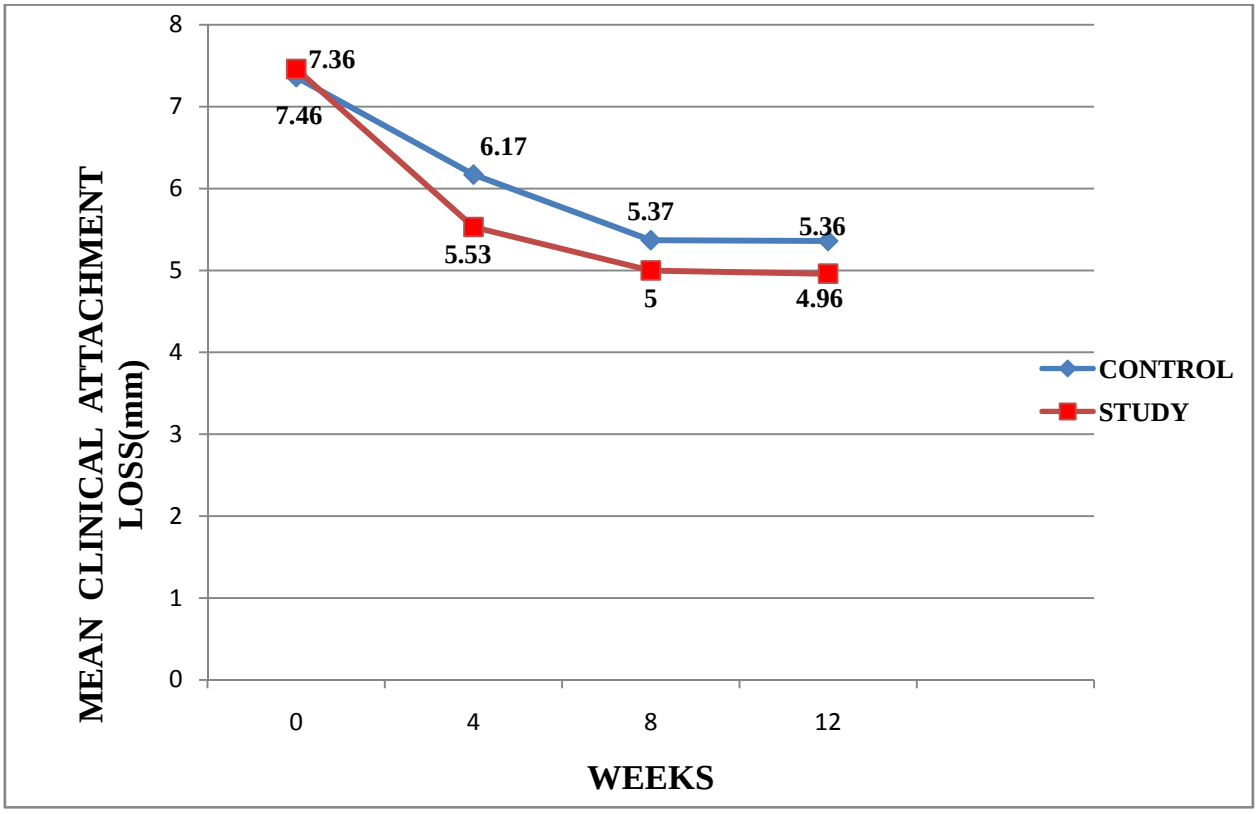


Figure-5 is the graphical representation of table-5.

TABLE-5A: MEAN CLINICAL ATTACHMENT LOSS(in mm)

BASELINE vs 8 WEEKS

	CONTROL GROUP	STUDY GROUP	p-VALUE
BASELINE	**7.36**	**7.46**	**= 0.62**
8 WEEKS	**5.37**	**5.00**	**<0.01**

Table-5A shows mean clinical attachment loss in both the groups at baseline and week 8.

- There was a statistically significant reduction in mean clinical attachment loss in the study group at week 8($p<0.01$).

FIGURE-5A: MEAN CLINICAL ATTACHMENT LOSS(in mm)

BASELINE vs. 8 WEEKS

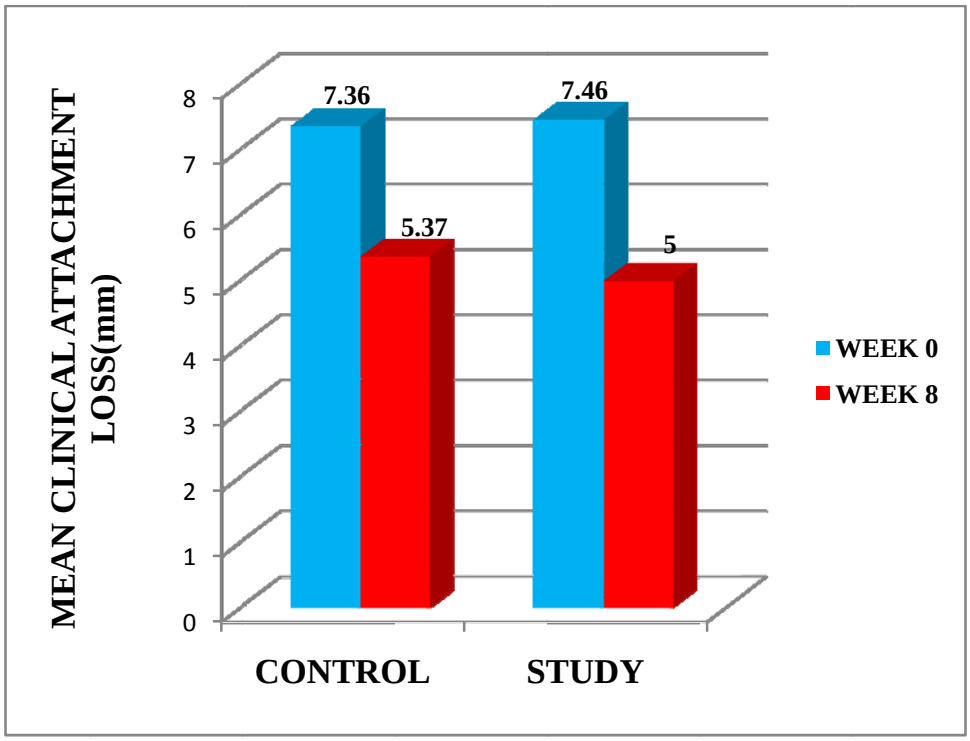


Figure-5A is the graphical representation of table-5A.

TABLE-6: MEAN ORAL HYGIENE INDEX:

	CONTROL GROUP		STUDY GROUP		INDEPENDENT T-TEST
	MEAN	SD	MEAN	SD	
BASELINE	1.99	0.50	1.96	0.49	p=0.83
WEEK 4	1.35	0.09	1.25	0.06	P<0.01
WEEK 8	1.51	0.18	1.38	0.12	P<0.01
WEEK 12	1.67	0.19	1.51	0.18	P<0.01
p-VALUE	p<0.01		p<0.01		

Table-6 shows mean oral hygiene index in both the groups from baseline to week 12.

- Statistical analysis within the group showed a significant reduction in mean oral hygiene index in both the control and study groups.
- Comparison between the groups showed statistically significant reduction in mean oral hygiene index from week 4 onwards.
- Post treatment follow up period at week 12 showed lesser mean oral hygiene index in the study group than the control group.

FIGURE-6 MEAN ORAL HYGIENE INDEX:

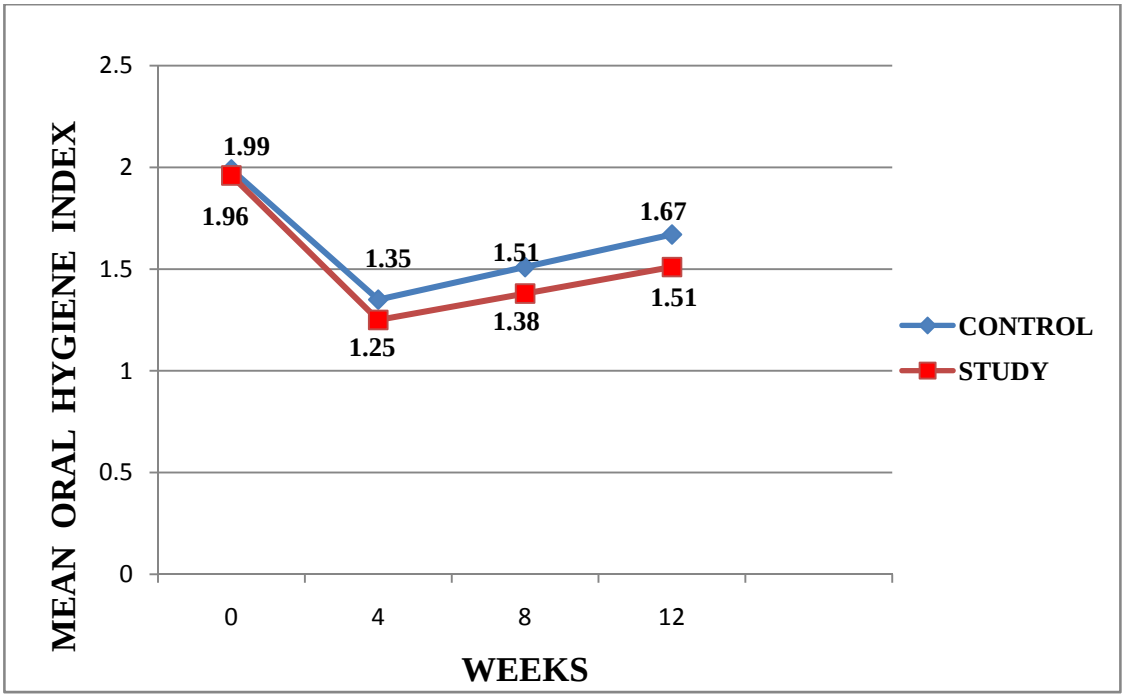


Figure-6 is the graphical representation of table-6.

TABLE-6A: MEAN ORAL HYGIENE INDEX

BASELINE vs 8 WEEKS

	CONTROL GROUP	STUDY GROUP	p-VALUE
BASELINE	**1.99**	**1.96**	**= 0.83**
8 WEEKS	**1.51**	**1.38**	**<0.01**

Table-6A shows mean oral hygiene index in both the groups at baseline and week 8.

- Comparison between the groups showed a statistically significant reduction in mean oral hygiene index at week 8 ($p < 0.01$).

FIGURE-6A: MEAN ORAL HYGIENE INDEX

BASELINE vs. 8 WEEKS

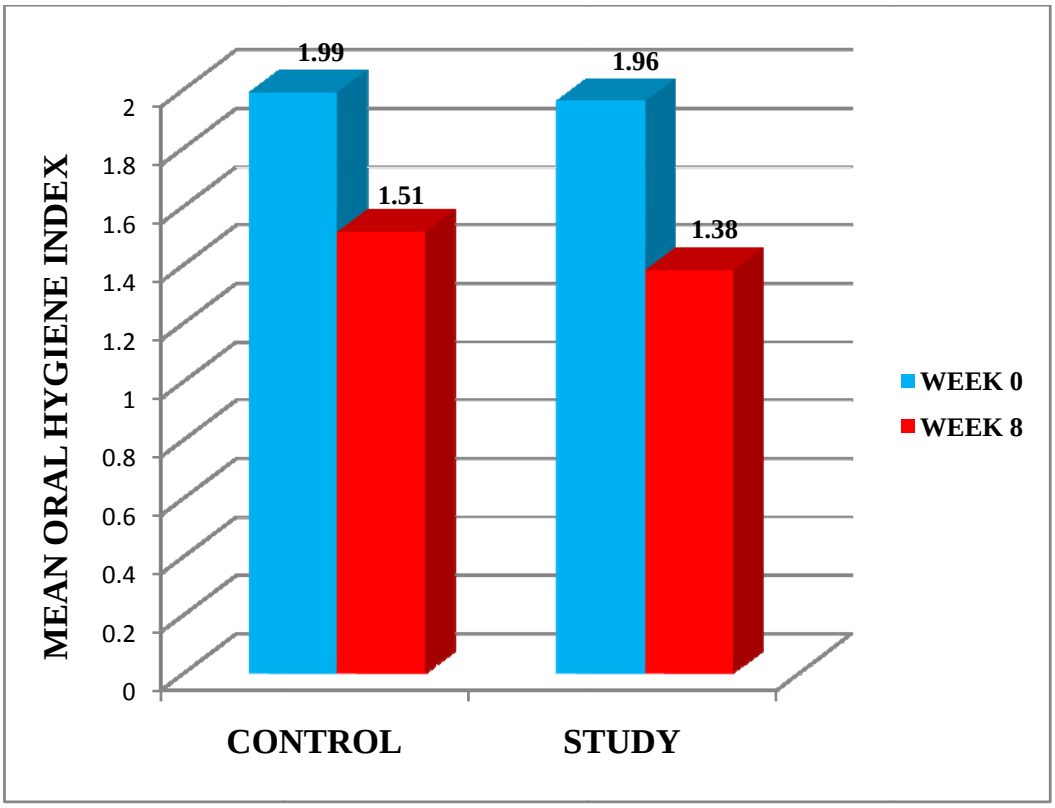


Figure-6A is the graphical representation of table-6A.

TABLE-7: MEAN GINGIVAL BLEEDING INDEX:

(BOP – Bleeding on Probing)

	CONTROL GROUP (NO.OF PATIENTS)		STUDY GROUP (NO.OF PATIENTS)		CHI-SQUARE TEST
	BOP POSITIVE	%	BOP POSITIVE	%	
BASELINE	30	100	30	100	p=0.83
WEEK 4	7	23	1	3	P<0.02
WEEK 8	6	20	0	0	P<0.01
WEEK 12	5	17	0	0	P<0.01
p-VALUE	p<0.01		p<0.01		

Table-7 shows mean gingival bleeding index in both groups from baseline to week 12.

- Statistical analysis within the group showed a significant reduction in mean gingival bleeding index in both the control and study groups.
- Comparison between the groups showed statistically significant reduction in mean gingival bleeding index from week 4 onwards.
- None of the patients had bleeding on probing at week 8 in the study group.

FIGURE -7: MEAN GINGIVAL BLEEDING INDEX:

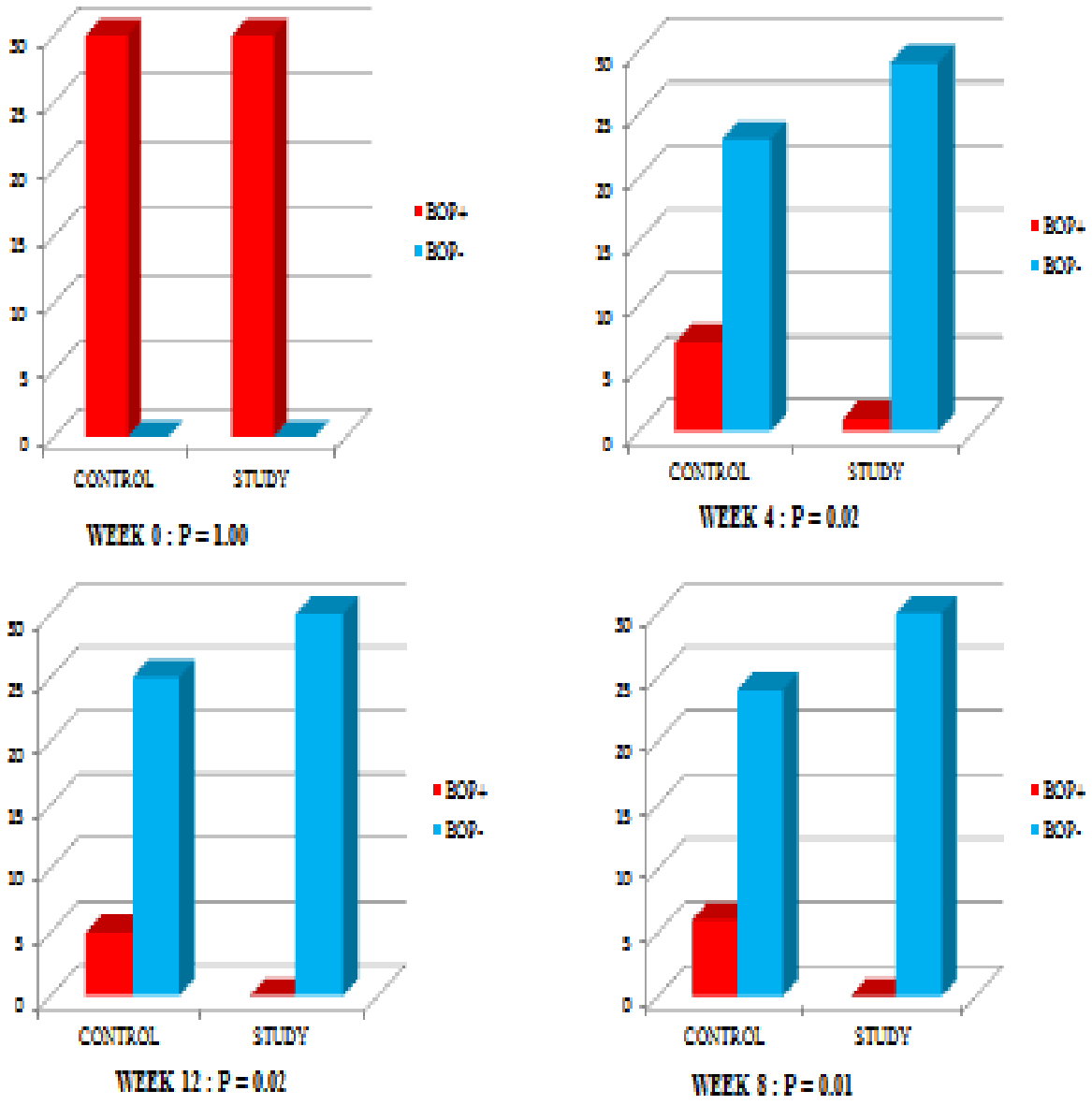


Figure-7 is the graphical representation of table-7.

BOP(+) means Bleeding on Probing present.

BOP(-)means Bleeding on Probing absent.

TABLE-8A : HAEMOGLOBIN LEVEL (g/dl)

GROUPS	BASELINE	8 WEEKS	p-VALUE
	MEAN (g/dl)	MEAN (g/dl)	
CONTROL	11.4	11.6	0.22
STUDY	11.5	11.6	0.18

Table 8A shows the mean haemoglobin level in both groups at baseline and 8 weeks.

- There was no statistically significant difference in the mean haemoglobin level in both groups at baseline and 8 weeks.

FIGURE-8A: HAEMOGLOBIN LEVEL (g/dl)

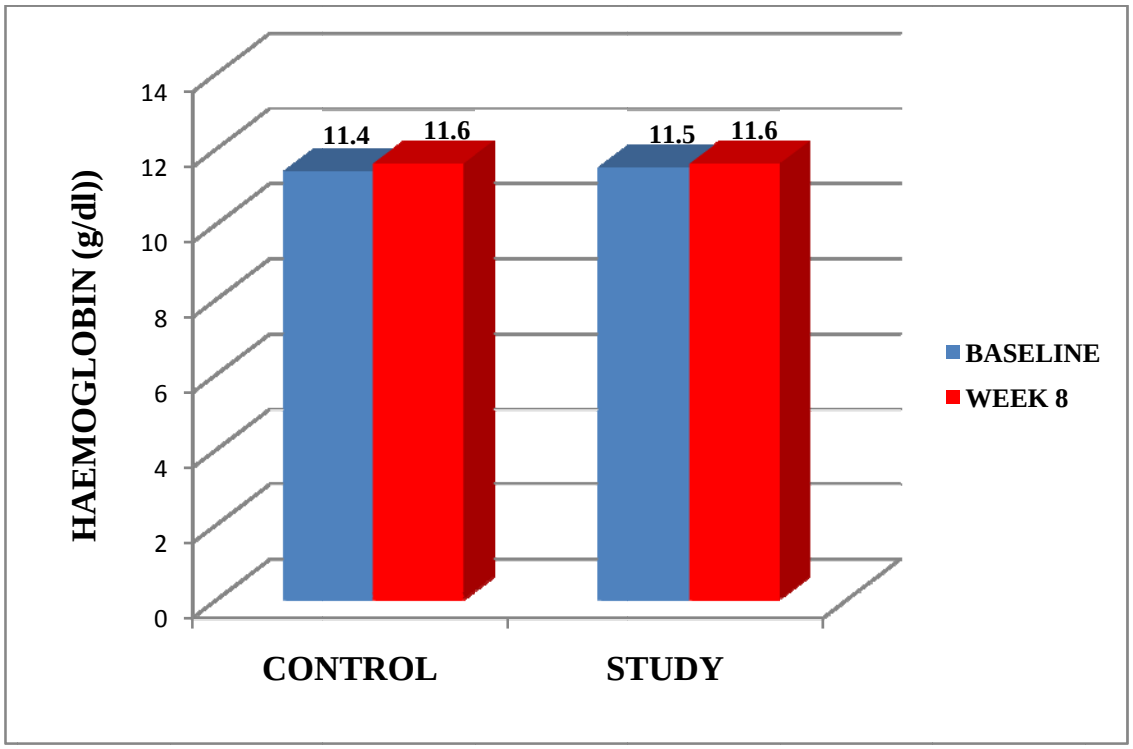


Figure-8A shows the mean haemoglobin value in control and study groups at baseline and 8 weeks.

TABLE-8B : TOTAL COUNT (cells/mm^3)

GROUPS	BASELINE	8 WEEKS	p-VALUE
	MEAN (cells/mm^3)	MEAN (cells/mm^3)	
CONTROL	8564	8463	0.69
STUDY	8916	8779	0.68

Table 8B shows the mean total count in both groups at baseline and 8 weeks.

- There was no statistically significant difference in the mean total count in both groups at baseline and 8 weeks.

FIGURE-8B: TOTAL COUNT(cells/mm^3)

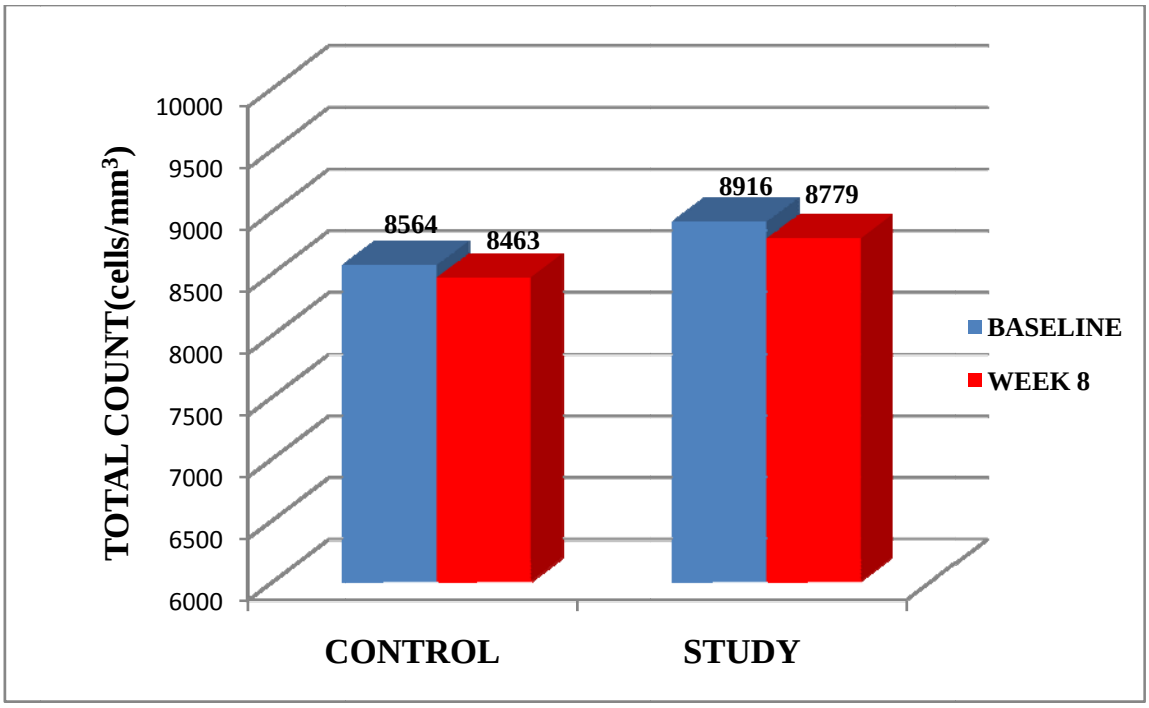


Figure-8B shows the mean total count value in control and study groups at baseline and 8 weeks

TABLE-8C : BLOOD SUGAR(mg/dl)

GROUPS	BASELINE	8 WEEKS	p-VALUE
	MEAN (mg/dl)	MEAN (mg/dl)	
CONTROL	95	94	0.80
STUDY	94	91	0.17

Table 8C shows the mean blood sugar value in both groups at baseline and 8 weeks.

- There was no statistically significant difference in the mean blood sugar value in both groups at baseline and 8 weeks.

FIGURE-8C: BLOOD SUGAR(mg/dl)

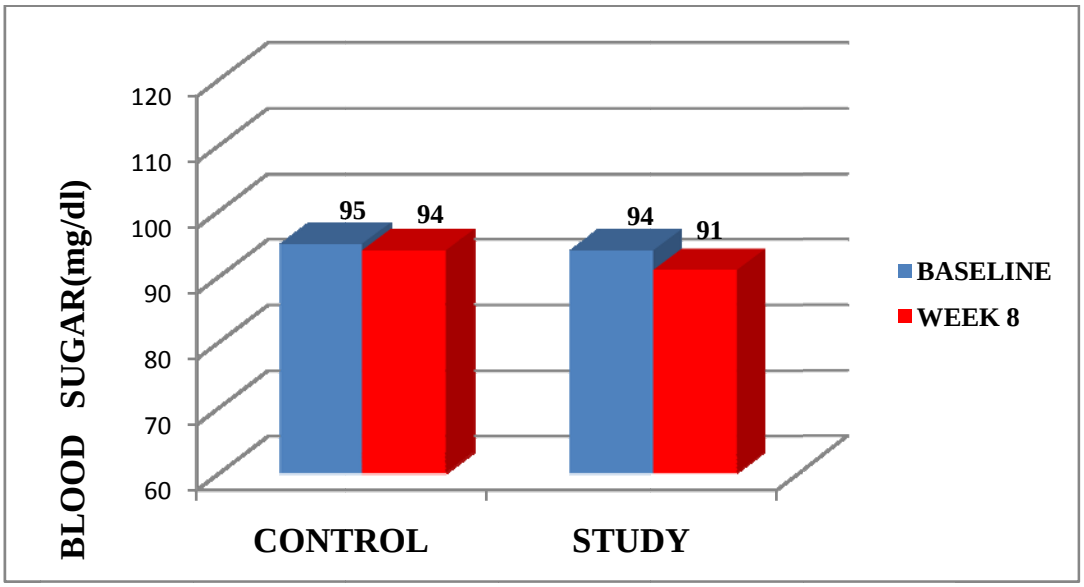


Figure-8C shows the mean blood sugar value in control and study groups at baseline and 8 weeks

TABLE-9: ADVERSE EVENTS

ADVERSE EVENTS	CONTROL GROUP	STUDY GROUP
NAUSEA	**3**	**2**
BLOATING	**3**	**4**
ABDOMEN PAIN	**3**	**2**
DIARRHOEA	**2**	**1**
HEAD ACHE	**1**	**1**

Table-9 shows the adverse events noted in both control and study groups.

- No significant difference was noted in the adverse events between the control and study groups.

FIGURE-9: ADVERSE EVENTS

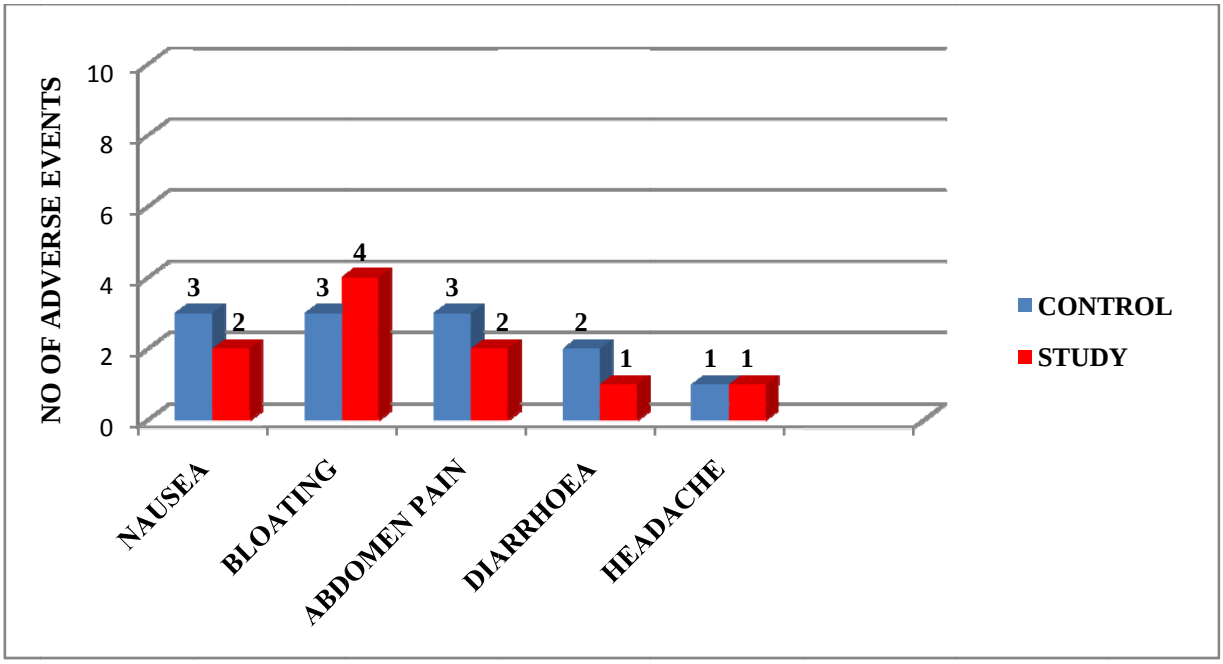


Figure-9 shows the adverse events noted during the study in graphical representation

FIGURE – 10: BEFORE TREATMENT

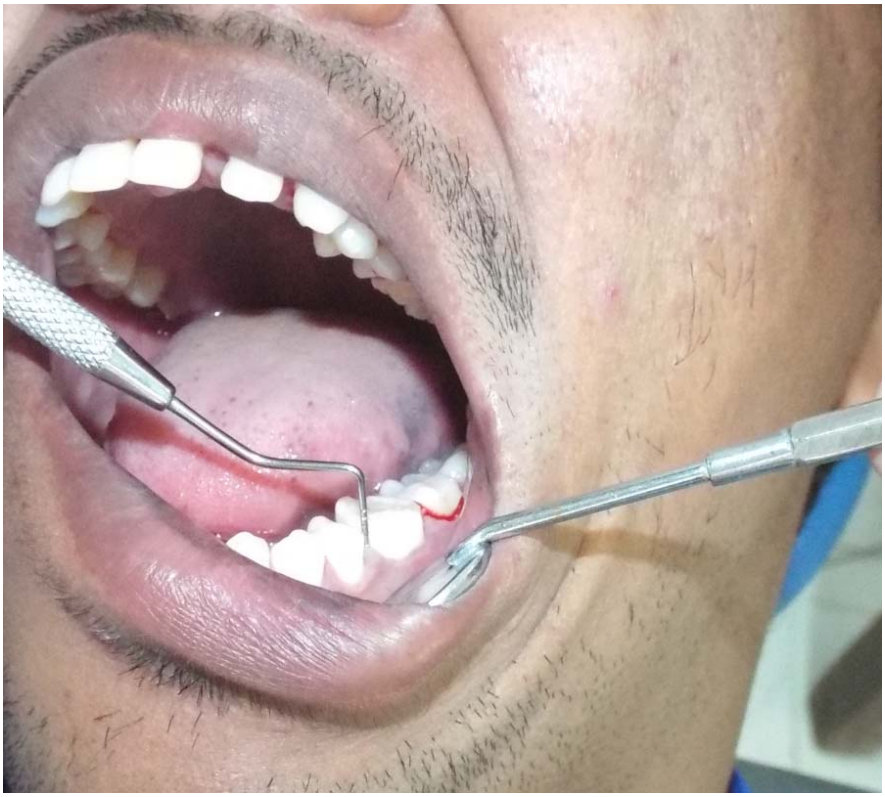


Figure 10 shows bleeding on probing and increased probing depth & clinical attachment loss.

FIGURE – 11: AFTER TREATMENT WITH SYNBIOTIC

Figure 11 shows no bleeding on probing and periodontal probe remains above the gingival margin due to decrease in probing depth & clinical attachment loss.

DISCUSSION

Aggressive periodontitis is characterized by rapid destruction of the periodontal ligament and alveolar bone around the affected teeth with increased probing depth formation and clinical attachment loss.

The standard therapy is Scaling and Root planing with systemic antibiotics. These antibiotics can lead to the emergence of drug resistant micro-organisms and also disturb the beneficial microflora of the body.

Hence, Synbiotics can be added, as they repopulate the beneficial microflora and reduce the pathogenic bacteria. The probiotics also produce different antimicrobial components which include hydrogen peroxide, organic acids, low-molecular weight antimicrobial substances and bacteriocins.

The use of probiotics was recently reported to significantly improve the periodontal health of subjects with aggressive periodontitis, a very destructive form of periodontitis (Shah et al. 2013).[8]

This study was done in the Institute of Pharmacology, Madras Medical College, Chennai in collaboration with the Department of Periodontics, Government Dental College, Chennai.106 patients were screened and 60 patients who fulfilled the inclusion criteria were enrolled for the study. They were randomized into 2 groups of 30 patients each.

Patients in the control group received the standard treatment in the form of Scaling and Root planing with Cap.Doxycycline 100mg twice daily for one week and Placebo onelozenge twice daily orallyfor a period of 8 weeks.Patients in the study group receivedthe standard treatment andSynbiotic one lozenge twice daily orally for a period of 8 weeks. Post treatment follow up was done for a period of 4 weeks.

The efficacy of the treatment was assessed at 4, 8 and 12 weeks by using standardized tables for Probing depth[PD],Clinical attachment loss[CAL],Oral hygiene index[OHI] and Gingival bleeding index[GBI].

Tolerability of the drugs was assessed by laboratory investigations and monitoring of adverse events during the study period. The data were collected and the results were analyzed statistically.

There was no significant difference in the mean age and sex distribution in both the control and the study groups. The mean age distribution in both the groups was 23. Females were more in numbers than males (17:13 in control group and 18:12 in study group).Patients with generalized aggressive periodontitis were higher in both the groups (20/30 in control group and 21/30 in study group).This correlates well with the study conducted by Loe et al[5].

In this study there was a statistically significant reduction in the probing depth($p<0.01$) in the study group than the control group at 8 weeks as compared to

the findings at baseline. This is in correlation with the studies conducted by Shimauchi H et al (2008)[37], Vivekananda M et al (2010)[57] which also showed a statistically significant reduction in the probing depth.

There was a statistically significant reduction in the Clinical attachment loss ($p<0.01$) in the study group than the control group at 8 weeks as compared to the findings on day 0. This correlates well with the studies conducted by Mayanagi et al (2009)[38], Vivekananda M et al (2010)[57] which also showed a statistically significant reduction in the Clinical attachment loss.

Our study showed a statistically significant decrease ($p<0.01$) in the Oral hygiene index in the study group than the control group at 8 weeks as compared to the findings on day 0. This was similar to the study done by Krasse P et al (2006)[39], Kang M et al (2006)[58], Riccia D et al (2007)[59] which also showed a statistically significant reduction in the Oral hygiene index.

There was a statistically significant reduction in the Gingival bleeding index ($p<0.01$) in the study group than the control group at 8 weeks as compared to the findings on day 0. This is in correlation with the studies conducted by Krasse P et al (2006)[39], Riccia D et al (2007)[59], Tsubura S et al (2009)[60], Vivekananda M et al (2010)[57] which also showed a statistically significant reduction in the Gingival bleeding index.

Addition of synbiotics to standard treatment showed a statistically significant reduction in mean probing depth, mean clinical attachment loss, mean oral hygiene index and Gingival bleeding index when compared to standard treatment alone. This correlates well with the studies conducted by Mishal Piyush Shah et al (2013)[8].

There was no statistically significant difference in the haematological parameters like Hemoglobin and Total WBC count and biochemical parameter like Blood Sugar in both the control and study groups at the end of treatment period when compared with the baseline. This study showed that synbiotic did not have any effect in the haematological and biochemical lab parameters.

No serious adverse events were reported in our study. Abdomen pain, Bloating and Nausea were the common adverse events reported during the study period in both the groups. Other adverse events noted were diarrhea and headache. This suggests that addition of synbiotic is not associated with increase in incidence of any adverse events, thereby showing the safety of synbiotic. Similarly the safety of synbiotic was also well established in a study done by Chatterjee et al (2011)[52].

Thus synbiotic can be used in patients with aggressive periodontitis and its use can improve the periodontal health. Synbiotic was not associated with any serious adverse effects and hence can be safely used in the management of aggressive periodontitis.

CONCLUSION

From this study, we conclude that

- Synbiotic when added to Standard treatment is more efficacious than Standard treatment alone in patients with Aggressive Periodontitis.
- Synbiotic is well tolerated and is not associated with serious adverse events.

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APPENDIX - I

LIST OF ABBREVIATIONS USED

AAP	–	American Academy of Periodontology
ANOVA	–	Analysis of Variance.
CAL	–	Clinical Attachment Loss or Level
CEJ	–	Cemento Enamel Junction
CI	–	Calculus Index
DI	–	Debris Index
GAP	–	Generalized Aggressive Periodontitis
GRAS	–	Generally Regarded as Safe
HLA	–	Human Leukocyte Antigens

LAP	–	Localized Aggressive Periodontitis
LPS	–	Lipopolysaccharide
MHC	–	Major Histocompatibility Complex
OEI	–	Oligofructose-Enriched Inulin
OHI	–	Oral Hygiene Index
PD	–	Probing Depth
PMNs	–	Polymorphonuclear leukocytes
SRP	–	Scaling and Root Planing
TGF	–	Transforming Growth Factor.
VSC	–	Volatile Sulphur Compounds

APPENDIX - II

ACOMPARATIVE STUDY OF SYNBIOTIC AS AN ADD ON THERAPY TO STANDARD TREATMENT IN PATIENTS WITH AGGRESSIVE PERIODONTITIS

CASE REPORT FORM

NAME: AGE/SEX : PLACE:

OP No: DIAGNOSIS:

Inclusion criteria: **YES/NO**

- Age: 18-30 years
- Sex-both genders
- Patients recently diagnosed with aggressive periodontitis
- Patients willing to give written informed consent and come for follow up
- Patients adhering to oral hygiene instructions and education.

Exclusion criteria: **YES/NO**

- Smokers and Alcoholics
- Pregnant and lactating women
- Patients treated with pre / probiotics in the last one month
- Patients allergic to prebiotics/probiotics
- Participation in another clinical study in the last three months
- Patients with Diabetes, Hypertension and chronic systemic illness of liver, heart, Kidney, GIT, CNS and HIV infection/AIDS.

Subject initials: Subject number:

Subject : Included/Excluded Reason if excluded:

Informed Consent Obtained: Yes/No

CONTROL/ TEST

Subject initials:

Subject number:

Signature of principal investigator

VISIT 1

1. Vitals:
2. Medical and Dental History:
3. General /systemic and Local (intra oral) examination:
4. Investigations:

   Hb: Total WBC count: Differential count:

   Blood sugar:

   X-ray -Intra Oral Peri-apical radiograph / Orthopantamogram
5. Oral hygiene index
6. Gingival bleeding index
7. Probing depth
8. Clinical attachment loss

VISIT 2

1. Vitals:
2. Adverse Events:

VISIT 3

1. Vitals:
2. Oral hygiene index

3. Gingival bleeding index
4. Probing depth
5. Clinical attachment loss
6. Adverse Events:

VISIT 4

1. Vitals:
2. Adverse Events:

VISIT 5

1. Vitals:
2. Oral hygiene index
3. Gingival bleeding index
4. Probing depth
5. Clinical attachment loss
6. Adverse Events:
7. Investigations:

   Hb: Total WBC count: Differential count:

   Blood sugar:

VISIT 6

1. Vitals:
2. Oral hygiene index
3. Gingival bleeding index

4. Probing depth
5. Clinical attachment loss

ASSESSMENT

I.Oral Hygiene Index=Debris index+Calculus index:

Debris index-score:

16	11	26
46	31	36

DI-S=

Calculus index-score:

16	11	26
46	31	36

CI-S=

II.Gingival Bleeding Index:

18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28
48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38

III.ProbingDepth(PD) & Clinical Attachment Loss(CAL) (in mm):

Maxillary:

Palatal

CAL																
PD																
	18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28
PD																
CAL																

Buccal

Mandibular:

Lingual

CAL																
PD																
	48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38
PD																
CAL																

Buccal

APPENDIX - III

PATIENT INFORMATION SHEET

Title:**A COMPARATIVE STUDY OF SYNBIOTIC AS AN ADD ON THERAPY TO STANDARD TREATMENT IN PATIENTS WITH AGGRESSIVE PERIODONTITIS**

Investigator:

Name of Participant:

This study is conducted at the Department of Periodontics, Govt.Dental College & Hospital, Chennai. You are invited to take part in this study. The information in this document is meant to help you decide whether or not to take part. Please feel free to ask if you have any queries or concerns.

Purpose of this study

Aggressive Periodontitis causes rapid destruction of tooth-supporting soft and hard tissues of the periodontium.Treatment must be pursued with a logical and regimented approach.In the recent times when organisms are fast developing resistance to antibiotics,the emergence of probiotics appears to be a boon in the treatment of periodontitis along with the conventional periodontal therapy. In this study we want to evaluate the safety and efficacy of synbiotics as an add on therapy to scaling and root planing(SRP).We have obtained permission from the Institutional Ethics Committee.

Study details

All patients in the study will be randomly allocated to 2 groups- A & B and will be given the respective treatment for a period of 8 weeks.

Study Procedures

During this study, blood will be collected from you at the beginning of the study. The total amount of blood collected from you will not be more than 7ml. You will be asked to come for follow up twice after 8 weeks of completion of study. During the course of the study if you notice any adverse events, you have to report it. You will be required to return unused study medicines when you report for your scheduled visits. This will enable correct assessment of the study results.

Possible benefits to you – Synbiotics with your standard medications will provide a better clinical outcome particularly in terms of Probing depth reduction & Attachment level gain than SRP alone and will reduce your future risk of developing complications due to Aggressive Periodontitis.

Possible benefits to other people - The results of the research may provide benefits to the society in terms of advancement of medical knowledge and/or therapeutic benefit to future patients.

Confidentiality of the information obtained from you

You have the right to confidentiality regarding the privacy of your medical information (personal details, results of physical examinations, investigations, and your medical history). By signing this document, you will be allowing the research team investigators, other study personnel, sponsors, Institutional Ethics Committee and any person or agency required by law like the Drug Controller General of India to view your data, if required.The information from this study, if published in scientific journals or presented at scientific meetings, will not reveal your identity.

Participation and Withdrawal from the study

Your decision not to participate in this research study will not affect your medical care or your relationship with the investigator or the institution. You will be taken care of and you will not lose any benefits to which you are entitled. The participation in this research is purely voluntary and you have the right to withdraw from this study at any time during the course of the study without giving any reasons. However, it is advisable that you talk to the research team prior to stopping the treatment/discontinuing of procedures etc.The results of this study will be informed to you at the end of the study.

Signature of Investigator Signature of Participant

Date: Date:

APPENDIX - V

INFORMED CONSENT FORM

Title: A COMPARATIVE STUDY OF SYNBIOTIC AS AN ADD ON THERAPY TO STANDARD TREATMENT IN PATIENTS WITH AGGRESSIVE PERIODONTITIS

Name of the Participant:

I _____ have read the information in this form (or it has been read to me). I was free to ask any questions and they have been answered. I am over 18 years of age and, exercising my free power of choice, hereby give my consent to be included as a participant in this study.

1. I have read and understood this consent form and the information provided to me.
2. I have had the consent document explained to me.
3. I have been explained about the nature of the study.
4. I have been explained about my rights and responsibilities by the investigator.
5. I am aware of the fact that I can opt out of the study at any time without having to give any reasonand this will not affect my future treatment in this hospital.
6. I hereby give permission to the investigators to release the information obtained from me as resultof participation in this study to the sponsors, regulatory authorities, Govt. agencies, and IEC.Iunderstand that they are publicly presented.
7. I have understand that my identity will be kept confidential if my data are publicly presented
8. I have had my questions answered to my satisfaction.
9. I have decided to be in the research study.

I am aware that if I have any question during this study, I should contact the investigator. By signingthis consent form I attest that the information given in this document has been clearly explained to meand understood by me, I will be given a copy of this consent document.

Name and signature / thumb impression of the participant (or legal representative if participantincompetent)

Name _____ Signature_____ Date_____

Name and Signature of impartial witness (required for illiterate patients):

Name _____ Signature_____ Date_____

Address and contact number of the impartial witness:

Name and Signature of the investigator or his representative obtaining consent:

Name _____ Signature_____ Date_____