EVALUATION OF SYSTEMIC MARKERS RELATED TO ANEMIA IN THE PERIPHERAL BLOOD OF GENERALIZED AGGRESSIVE PERIODONTITIS PATIENTS BEFORE AND AFTER PHASE I PERIODONTAL THERAPY - AN INTERVENTIONAL STUDY

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ADHIPARASAKTHI DENTAL COLLEGE AND HOSPITAL

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CERTIFICATE

This is to certify that **Dr. P. SHOBANA**, Post graduate student (2015-2018) in the Department of Periodontics, Adhiparasakthi Dental College and Hospital, Melmaruvathur – 603319, has done this dissertation titled **"EVALUATION OF SYSTEMIC MARKERS RELATED TO ANEMIA IN THE PERIPHERAL BLOOD OF GENERALIZED AGGRESSIVE PERIODONTITIS PATIENTS BEFORE AND AFTER PHASE I PERIODONTAL THERAPY- AN INTERVENTIONAL STUDY"** under our direct guidance and supervision in partial fulfillment of the regulations laid down by The Tamilnadu Dr. M.G.R Medical University, Chennai – 600032 for MDS; (Branch II) Department of Periodontics Degree Examination.

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DECLARATION

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I hereby declare that no part of the dissertation will be utilized for gaining financial assistance or any promotion without obtaining prior permission of the Principal, Adhiparasakthi Dental College and Hospital, Melmaruvathur – 603319. In addition, I declare that no part of this work will be published either in print or in electronic media without the guides Knowledge, who have been actively involved in dissertation. The author has the right to reserve for publish work solely with the permission of the Principal, Adhiparasakthi Dental College and Hospital, Melmaruvathur – 603319

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ABSTRACT

BACKGROUND:

Periodontal infections like Chronic and Aggressive type of periodontitis were found to be associated with systemic diseases. Studies suggested that it might enhance the risk for certain systemic diseases. Like vice, some systemic conditions has its effect on the periodontal health. Diabetes mellitus tend to increase the risk for periodontal disease and vice versa. Recently many researchers suggested the link between chronic periodontitis and anemia. The association of chronic periodontitis with decreased red blood cell parameters suggested that this condition may be linked with Anemia of Chronic Disease. Very few studies explored the effect of Aggressive periodontitis on erythrocyte counts and hemoglobin levels. This is the first interventional study to investigate the hematological parameters in Generalized Aggressive Periodontitis patients and also evaluate the effect of Non-surgical periodontal therapy on blood parameters of Generalized Aggressive Periodontitis patients.

AIM AND OBJECTIVE:

The main aim of this study is to evaluate the systemic markers related to Anemia in Generalized aggressive periodontitis patients before and after phase I periodontal therapy.

MATERIAL AND METHODS

A total number of 30 young adults diagnosed with Generalised Aggressive Periodontitis were selected for the study and after obtaining sign in a written informed consent clinical parameters such as Plaque Index, Gingival bleeding index, Probing depth, Clinical attachment level and Blood parameters viz., RBC, Hb, PCV, MCV, MCH, MCHC, ESR and Serum Ferritin levels estimation were done. Based on the inclusion criteria 17 patients with serum ferritin level above 30ng/dl were enrolled in the study, of which two patients discontinued. Finally 15 patients were considered and subjected to

phase I periodontal therapy. At the end of three months clinical parameters and hematological parameters were re-evaluated. The obtained results were statistically analysed using paired *t*- test and Wilcoxon signed rank test.

RESULTS:

The Hematological parameters such as Hemoglobin and RBC count were found to be increased significantly after phase I therapy with a significant improvement in the Plaque Index score, Gingival bleeding index score, reduction of Probing depth and gain in Clinical attachment levels. The other blood parameters viz., PCV, MCV, MCH, MCHC values were minimally increased after phase I therapy but the difference was not found to be statistically significant in this study. The ESR value also significantly decreased post-operatively.

CONCLUSION:

Within the limitation of this present interventional study it could be concluded that Generalized Aggressive Periodontitis was associated with reduced Red blood cell parameters suggesting that it may tend toward anemia of chronic disease. The treatment by Non-surgical periodontal therapy not only reverses the periodontal health by reducing the inflammation but also improves the anemic status.

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ACD	**:**	**Anemia of Chronic Disease**
BOP	**:**	**Bleeding on Probing**
BT	**:**	**Bleeding Time**
CAL	**:**	**Clinical Attachment Level**
CD	**:**	**Cluster of Differentiation**
CEJ	**:**	**Cementoenamel junction**
CGP	**:**	**Chronic generalized periodontitis**
CP	**:**	**Chronic Periodontitis**
CKD	**:**	**Chronic kidney disease**
CRP	**:**	**C- Reactive proteins**
CT	**:**	**Clotting time**
ESR	**:**	**Erythrocyte sedimentation rate**
EDTA	**:**	**Ethylene di-amine tetra acetic acid**
EPO	**:**	**Erythropoeitin**
GBI	**:**	**Gingival Bleeding index**
GAgP	**:**	**Generalized Aggressive Periodontitis**
GI	**:**	**Gingival index**
Hb	**:**	**Hemoglobin**
HCT	**:**	**Haematocrit**
HLA	**:**	**Human leucocyte antigen**
Ig	**:**	**Immunoglobulin**
IL	**:**	**Interleukin**

INF	**:**	**Interferon**
JIA	**:**	**Juvenile idiopathic arthritis**
LAP	**:**	**Localised Aggressive Periodontitis**
MCV	**:**	**Mean corpuscular volume**
MCH	**:**	**Mean corpuscular Haemoglobin**
MCHC	**:**	**Mean corpuscular haemoglobin concentration**
NSPT	**:**	**Non-surgical periodontal therapy**
PI	**:**	**Plaque index**
PD	**:**	**Probing Depth**
PCV	**:**	**Packed cell volume**
PMN	**:**	**Polymorphonuclear neutrophils**
RA	**:**	**Rheumatoid arthritis**
RBC	**:**	**Red blood cell count**
SRP	**:**	**Subgingival scaling and root planing**
SI	**:**	**Serum Iron**
TIBC	**:**	**Total Iron binding capacity**
TLC	**:**	**Total leucocyte count**
TNF	**:**	**Tumor necrosis factor**
WBC	**:**	**White blood cells**

INTRODUCTION

Anemia is been defined as a decrease in the number of erythrocyte count or in the concentration of hemoglobin which results in a reduction in the oxygen carrying capacity of blood.[1] In other terms it can be defined as a "condition in which the blood is deficient either in quantity or in quality" The deficiency in quality may consist in diminution of the number of RBCs or the hemoglobin or both, it may be local or due to mechanical interference with the supply of blood or general. Whereas, the deficiency in quantity or quality is due to imperfect nutrition, wasting disease or direct loss of blood.[2] Anemia is considered as a major health problem affecting both developing and developed countries and an indicator of poor nourishment that significantly affects not only the human health but also the social well-being and economic development. Thus, it is an important contributing factor to the global burden of disease. Most common cause of anemia is iron deficiency. The other possible cause could be chronic infections, inflammatory conditions and micronutrient deficiency. Anemia caused by chronic infection or inflammation are described as Anemia of chronic disease (ACD) and are the second most prevalent after iron deficiency anemia.[3]

Anemia of chronic disease (ACD) has been defined as the anemia occurring in chronic inflammatory conditions which are not due to marrow deficiencies or other diseases and occurring despite in the presence of adequate iron stores and vitamins. [4]

Hutter *et al*., 2001 stated that periodontitis is the most common inflammatory diseases of humans, like other chronic conditions leads to anemia.[5] Periodontitis is an inflammatory disease of the supporting structures of the teeth which is characterized by progressive destruction of the periodontium initiated by chronic bacterial infections.[6] Though, the tissue destruction in periodontitis is confined to the periodontium, the microorganisms or their products can invade the periodontal tissues through the ulcerated pocket epithelium and gets access to reach the systemic circulation.[7] A large number of studies have proved the potential relationship between periodontal disease and several systemic conditions like cardiovascular disease, diabetes mellitus, adverse pregnancy outcomes, obesity etc[8]. Researchers also have suggested that periodontitis patients have an altered blood cell counts when compared with periodontally healthy controls. In an anemic state it was conceivable that lack of oxygen to the tissues acted as a modifying factor in the response of periodontium to local irritation [2]. Studies have supported the hypothesis that severe periodontitis might lead to Anemia and also provided evidence that non-surgical periodontal therapy tend to improve the status of anemia.

Various studies have correlated the presence of Anemia with chronic periodontitis. Lesser document is available for inter-relationship between anemia and aggressive periodontitis. Aggressive periodontitis can be distinguished from chronic periodontitis by the rapid rate of destruction, age of onset of disease, alteration in the host

immune response, composition of the subgingival microbiota, familial aggregation and a strong racial influence.

Baer 1971 defined Aggressive periodontitis as a disease of the periodontium occurring in an otherwise healthy adolescent that is characterized by rapid loss of alveolar bone about more than one tooth of the permanent dentition. In the year 1989, the World Workshop in Clinical Periodontics described this as localized juvenile Periodontitis (LJP) and as a subset of the broad classification of early-onset periodontitis (EOP). Later in 1999, the World Workshop in Periodontics introduced a new classification system; several rapidly progressive periodontitis were united under the term Aggressive Periodontitis.[9] It can be either localized or generalized. Localized Aggressive Periodontitis (LAP) is commonly seen in younger adults, characterized as having localized first molar/incisors with interproximal attachment loss on atleast two permanent teeth one of which is a first molar. Generalized aggressive periodontitis (GAgP) is been characterized as having generalized interproximal attachment loss that found to be affecting atleast three permanent teeth other than first molars and incisors.

Anand *et al.*, 2014 suggested that generalized aggressive periodontitis like chronic periodontitis may be associated with risk for **Anemia of chronic disease**.[1] Treating Aggressive Periodontitis patients with Non-surgical periodontal therapy has an influence in reversing the

anemic status. Conventional phase I therapy consists of mechanical supra and subgingival tooth debridement and self-administered oral health care measure instructions. These measures help in reducing the bacterial load and altering the composition of subgingival microflora that are more beneficial to health.[10]

Very few studies are available at present to describe the relationship between Anemia and Aggressive periodontitis. This present study is undertaken to evaluate the systemic markers related to anemia in the peripheral blood of Aggressive periodontitis patients before and after Non-surgical Periodontal therapy.

AIM AND OBJECTIVES

The main aim of the study is to evaluate and compare the red blood cell parameters in Generalized Aggressive Periodontitis patients before and after Phase I periodontal therapy.

1) To investigate whether Generalized Aggressive Periodontitis is related with reduced erythrocyte count and reduced Hemoglobin levels,
2) To evaluate the effect of Non-surgical periodontal therapy on systemic markers related to Anemia in patients with Generalized Aggressive Periodontitis,
3) To compare and correlate the clinical parameters such as Plaque index, Gingival bleeding Index, Probing depth and Clinical attachment level with Hematological parameters in Generalized Aggressive Periodontitis patients at baseline and after therapy.

GENERAL REVIEW

ANAEMIA

DEFINITION: "It is a state in which the concentration of hemoglobin level in the blood is below the normal range for the age and gender of an individual". [11]

Determination of haematological parameters related to anaemia is an important way to early diagnose and treat the disease thereby eliminating its further complications. Even though the value of hemoglobin is the most commonly employed major parameter to determine whether anaemia is present or not the red cell counts (RBC), Hematocrit (PCV) and blood indices (viz., MCV, MCH, and MCHC) also equally provide an alternative means of assessing anaemia [12].

CLASSIFICATION OF ANEMIA: [12]

Anemia can be classified based on pathophysiologic mechanism and morphologic alteration of red blood cells.

PATHOPHYSIOLOGIC CLASSIFICATION:

1) ANEMIA DUE TO INCREASED LOSS OF BLOOD
    a) Acute post-haemorrhagic anaemia
    b) Chronic blood loss
2) ANEMIA DUE TO IMPAIRED PRODUCTION OF RED BLOOD CELLS

CYTOPLASMIC MATURATION DEFECT:

a) Deficient haem synthesis (iron deficiency anemia)

b) Deficient globin synthesis (Thalassemic syndromes)

NUCLEAR MATURATION DEFECT:

a) Vitamin B 12 or Folic acid deficiency

b) Megaloblastic anemia

c) Defect in stem cell proliferation and differentiation (aplastic anemia, pure red cell aplasia)

d) **ANEMIA OF CHRONIC DISEASE**

e) Bone marrow infiltration

f) Congenital anemia

3) ANEMIA DUE TO INCREASED RED CELL DESTRUCTION

a) Haemolytic anemia

MORPHOLOGIC CLASSIFICATION:

1) MICROCYTIC, HYPOCHROMIC
2) NORMOCYTIC, NORMOCHROMIC
3) MACROCYTIC, NORMOCHROMIC

ANEMIA OF CHRONIC DISEASE:

Anemia of chronic disease (ACD) is a commonly occurring poorly understood condition that is encountered in patients with a variety of diseases, particularly in hospital population including chronic infections, malignancies and rheumatological diseases. [13]

Schilling specified that the term "Anemia of chronic disease" is a misnomer and it has to be replaced with "Anemia of inflammation" because chronic may be misleading because changes in hemoglobin levels and hematocrit can occur in less than 2 weeks. Many authors preferred to use the term "Anemia of chronic disease" because of the multifactorial and complex etiology of this disease. [14]

This type of anemia is not related to bleeding, hemolysis or actual invasion of bone marrow, it develops secondary to an underlying disease process. The severity of anemia is directly related to the primary disease process and can be corrected only if the primary disease is alleviated. In general the peripheral blood picture shows ACD as Normocytic and Normochromic but can have mild degree of microcytosis and hypochromia in long standing inflammation that is unrelated to iron deficiency [11, 12]. It is characterised by reduced serum iron and total iron binding capacity but iron stores are normal or increased as indicated by the serum ferritin or prusian blue stain for marrow iron. [15]

Conditions that commonly associated with ACD are acute / chronic infections, autoimmune disorders, chronic kidney diseases (CKD) and chronic inflammatory conditions.

The prevalence range of ACD caused due to chronic inflammation is 23-50%, thus it was also termed as "Anemia of inflammation" (AoI). [13]

Cartwright postulated that three pathologic processes found to be involved in the etiology of ACD: [15]

1) Shortened or decreased survival of Erythrocyte
2) Failure of bone marrow in the increased production of RBC to compensate for increased demand (defective RBC production)
3) Impaired release of iron from the reticuloendothelial system.

The other different pathways that have been presumed to cause ACD are aversion of iron traffic, reduced erythropoiesis, decreased response to erythropoietin, erythophagocytosis and invasion of bone marrow by tumour cells and pathogens.[13]

ANEMIA SECONDARY TO CHRONIC SYSTEMIC DISORDERS:[12]

The possible causes of anemia of chronic disease are as follows.,

1) Anemia of chronic inflammation or infection:
    a) Infections e.g. Tuberculosis, lung abscess, pneumonia, osteomyelitis, subacute bacterial endocarditis, pyelonephritis.
    b) Non-infectious inflammations e.g. autoimmune diseases like Rheumatoid arthritis, systemic lupus erythematosis, vasculitis, dermatomyositis, scleroderma, sarcoidosis, crohn's disease.

2) Anemia of renal disease:

   Uraemia, Renal failure.

3) Anemia of hypometabolic state:

   Protein malnutrition, Scurvy, Pregnancy, Liver disease, Endocrinopathies etc.

PATHOPHYSIOLOGY:

The etiology of ACD is categorized by an activation of immune cell and response of inflammatory cytokine that blunts erythropoietin production, decreases erythropoiesis, impaired red cell life span and dysregulated iron homeostasis [13].

1. DEFECTIVE RED CELL PRODUCTION:

Even though there is abundance of storage iron (serum ferritin) but the amount of iron in developing erythroid cells in the marrow is below normal. This is because of the mononuclear phagocyte system is hyperplastic and also traps all the available free iron due to hyperactivity of iron binding protein Lactoferrin. A defective transfer of iron from macrophages to the developing erythroid cells in the marrow leads to decreased availability of iron for haem synthesis despite of adequate iron stores, elevating serum ferritin levels. The defect lies in suppression by cytokines at some stage in erythropoiesis for e.g TNF, IFN-β released in bacterial infections and tumours, IL-1, IFN-γ released in patients of rheumatoid arthritis and autoimmune vasculitis. [12]

2. ALTERED EPO (ERYTHROPOIETIN) METABOLISM:

Erythropoietin is the primary hormone that is responsible for the regulation of erythropoiesis, in case of ACD it is inversely related with the haemoglobin. As Hb decreases EPO increases. Since it has a major role in erythropoiesis EPO has been a focus of investigation in ACD. Anemia associated with rheumatoid arthritis is the best example of ACD, investigation of EPO levels in ACD were performed in patients suffering from Rheumatoid arthritis. Increased serum EPO in response to the development of anemia was seen. Although these patients had increased serum EPO levels in response to development of anemia, the EPO levels were lower in anemic patients without rheumatoid arthritis. Decrease in the incremental response of EPO to anemia may be the cause of reduced erythropiesis in ACD but it cannot be taken into consideration as a primary cause because EPO levels are still seen to be higher in persons without anemia. Thus, the defective marrow response to these increases in EPO must be considered as the primary reason for the anemia.[15]

DECREASED EPO SYNTHESIS: EPO release in response to anemia is said to be blunted in patients with Anemia of Inflammation (AoI). This may be the result of the action of several cytokines.

IMPAIRED EPO ACTION: Decreased response of the erythroid precursors to EPO, this unresponsiveness may be the result of macrophage- derived factors.

3. DECREASED RBC SURVIVAL: [12, 14]

The life span of RBC is reduced to 60-90 days compared to the normal 90-120 days; it is mainly because of the role of macrophages in RBC disposal. Macrophage normal function is to remove senescent RBCs from the circulation; therefore it is possible that macrophages activated by the inflammatory process will exaggerate this regular function. Senescent RBCs and those coated by Igs or immune complexes are released efficiently by an activated phagocytic system.

ROLE OF SPECIFIC CYTOKINES IN THE ETIOPATHOGENESIS OF ACD:

CYTOKINES such as Interleukin-1 (IL-1), Interleukin-6 (IL-6), Tumour necrosis factor- α (TNF-α), and interferons were hypothesized to be involved in the maintenance of RBC production or stability.[16] **Means and krantz** stated that cytokines involved in the inflammatory response are found to be increased in the diseases associated with ACD. TNF-α plays a significant role in inflammatory and immune response. Administration of TNF to animals resulted in the development of anemia but no decrease in platelets or granulocyte counts were seen. Interleukin 6 (IL-6) is a pluripotent cytokine with pro-inflammatory, hematopoietic and immunomodulatory effects **Atkins *et al,* 1995** [17]. Anemia associated with administration of rhIL-6 was rapid in onset, progressive and dose-dependent and the condition reversed quickly after cessation of the rhIL-6 therapy. IL-1 is a polypeptide that has a wide variety of actions in inflammation and

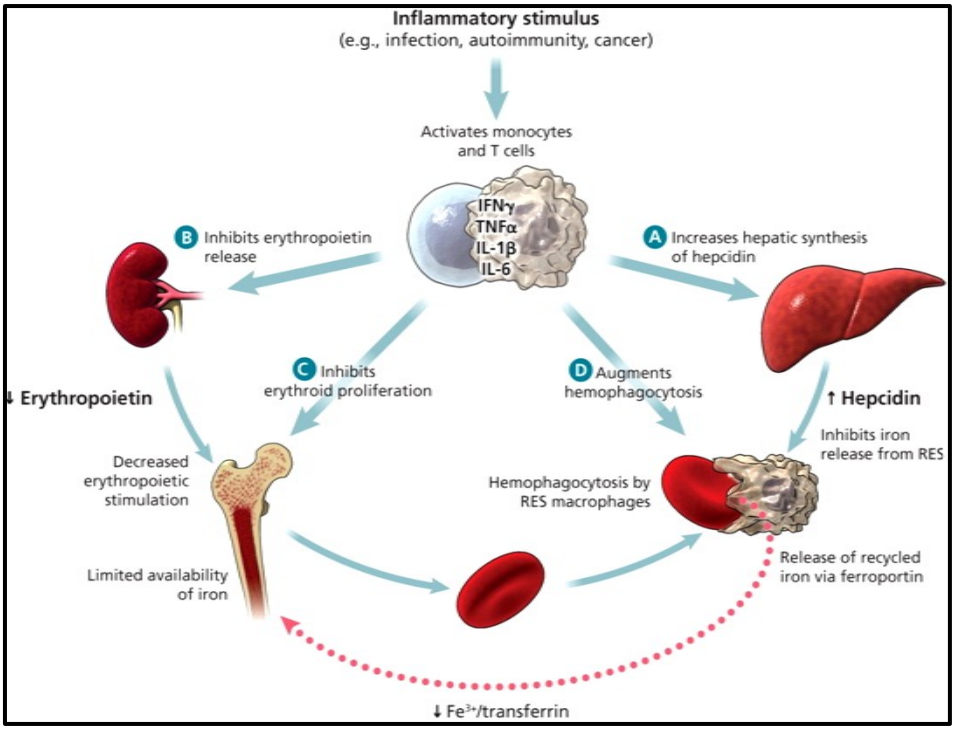
immunity and shares properties of TNF. IL-1 levels are elevated in Rheumatoid arthritis patients, this correlates with markers of disease activity such as anemia. γIFN is produced mainly by T- lymphocytes and is involved in the modulation of immune and inflammatory response as well as the host defence against microbial challenge. Increased levels of γ IFN seen in patients with autoimmune and infectious disease. Most of the cytokines involved in inflammatory and immune response inhibit erythroid colony formation in vitro or are associated with development of anemia including α and β IFNs, IL-6, TNF β and may merit investigation for a role in etiology of ACD.[15]

HEPCIDIN – HALLMARK IN ACD:[13]

Change in the pattern of iron distribution is initiated by inflammatory cytokines. ACD patients are found to have low serum iron, low or normal iron binding capacity, low transferrin saturation and low reticulocyte counts. An accumulation of iron in the reticuloendothelial macrophages despite of decreased circulating iron levels is the primary feature of ACD. Thus, reduced serum iron leads to reduced haemoglobin synthesis inspite of adequate iron storage. Recently researchers stated that Hepcidin has a functional role in iron deficiency present in ACD. **Weinstein** *et al.,* 2002[16] Hepcidin is a peptide hormone, produced in the liver and detectable in serum and urine. It has intrinsic antimicrobial activity and a response to inflammatory stimuli the expression of Hepcidin is increased. It is a negative regulator of Iron absorption in intestine and macrophage

associated iron release. **Interleukin 6 acts on the hepatocytes and induces the release of Hepcidin.**[13]

Hepcidin found to act by compounding and initiating the degradation of Ferroportin, which is the only known Iron exporter that is present on the surface of hepatocytes, duodenal enterocytes and macrophages thus it is one of the important transporters of cellular iron. Depletion of Ferroportin would stop the transfer of cellular iron into the plasma. Increased serum Hepcidin levels reduce the intestinal iron absorption and inhibit the export of iron from tissue storage into the blood stream. In contrast, the limited levels of iron in the circulation down regulates the synthesis of hepcidin, this allows an influx of bioavailable form of iron from duodenal enterocytes and tissue iron stores. Inflammation caused by infection, autoimmune disease and oral cancers stimulates the synthesis of pro-inflammatory cytokines such as INF, IL-1 and 1L-6 and these cytokines tend to induce the expression of excess Hepcidin, this has the ability to impair the function of ferroportin on duodenal enterocytes and macrophages that in turn impaired the absorption of iron from the gut and exaggerated iron retention. This mechanism of long term release of Hepcidin that causes ACD is said to be a Hall mark of ACD.



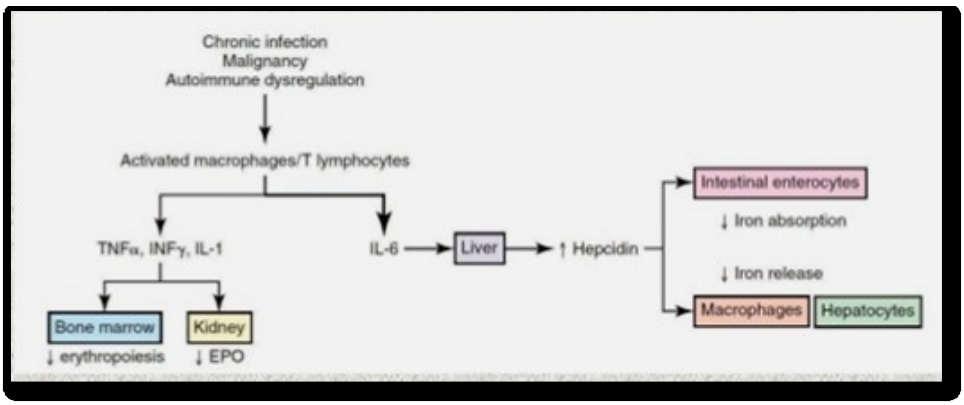
MECHANISM OF ACTION:[18] In case of inflammatory disease, cytokines released by the activation of leukocytes and other cells exert multiple effects that lead to the decrease in the level of haemoglobin.

(A) Induction of hepcidin synthesis in the liver especially by Interleukin-6 IL-6, hepcidine binds to ferroportin, the pore that allows egress of iron from reticuloendothelial macrophages and from intestinal epithelial cells. This binding of hepcidin to ferroportin leads to depletion of ferroportin; the corresponding sequestration of iron in the macrophage reduces the availability of iron to erythroid precursors.

(B) Inhibition of erythropoietin release from the kidney especially by Interleukin 1β (IL-1β) and TNF α, reduces the erythropoietin-stimulated hematopoietic proliferation.

(C) Direct inhibition of the proliferation of erythroid progenitors especially by TNF α, Interferon-γ [INFγ] and IL-1β.

(D) Augmentation of erythrophagocytosisbyreticuloendothelial macrophages.



HAEMATOLOGICAL DIFFERENCE BETWEEN IRON DEFICIENCY ANEMIA AND ACD:[13]

The indicator of serum iron stores are the levels of Transferrin. Initially it was called Siderophilin later; **Holmberg and Laurell** named it Transferrin. In Iron deficiency anemia Transferrin is found to be increased whereas Transferrin saturation is reduced. In ACD transferrin levels may remain normal or reduced. Transferrin saturation is reduced in ACD as well as in iron deficiency.

Ferritin is an acute phase reactant that is usually increased in chronic inflammation, autoimmune disorders, chronic infection and chronic liver diseases. It also plays a vital role in storage of iron and recycling. Ferritin molecule can store up to 4,500 iron atoms. The increase in the synthesis of ferritin during inflammation is because of the influence of IL-1 and TNF. Cytokines indirectly initiates ferritin synthesis by increasing the uptake of iron by Hepatocytes. Besides being an iron storage protein it also plays a critical role in macrophages where it recycles iron from old RBCs and transfers it to apoferritin. The iron present in transferrin is transported to immature RBCs in the bone marrow to complete the cycle.

A level of 15ng/ml of ferritin is generally indicative of absent iron stores. A predictive value of iron deficiency is reflected with ferritin levels 30ng/ml. In ACD, ferritin is increased due to immune activation along with increased storage and retention of iron within the reticuloendothelial system. The levels of soluble Transferrin receptor

is increased in iron deficiency even though the availability of iron for erythropoiesis is less. In contrast, the levels of soluble transferrin receptors in ACD are not affected because the inflammatory cytokines have a least impact on transferrin receptor expression. Soluble transferrin receptors evaluation is helpful in differentiating ACD patients and patients accompanied with both ACD and iron deficiency. The concentration ratio of soluble transferrin receptors to the log of ferritin levels may be useful in differentiating iron deficiency from ACD. A ratio of< 1 suggests ACD, whereas ratio more than 2 is an indicative of absolute iron deficiency co-existing with ACD.

Serum levels that differentiate Anemia of chronic disease from iron-deficiency anemia. (Guenterweiss 2005) [3]

VARIABLE	ANEMIA OF CHRONIC DISEASE	IRON DEFICIENCY ANEMIA	BOTH CONDITIONS
Iron	Reduced	Reduced	Reduced
Transferring	Reduced to normal	Increased	Reduced
Transferring saturation ferritin	Reduced	Reduced	Reduced
Ferritin	Normal or increased	Reduced	Reduced to normal
Soluble transferring receptor	Normal	Increased	Normal to increased
Ratio of soluble transferring receptor to log ferritin	Low (less than 1)	High (greater than 2)	High (>2)
Cytokine levels	Increased	Normal	Increased

MANAGEMENT OF ACD:[13]

The development of anemia would even more worsen the underlying chronic diseases. It is referred as a predictor of poor prognosis of that particular disease. An ideal treatment option for ACD is to eliminate/ reduce the underlying chronic disease process.

Therapeutic management of ACD includes increasing Hemoglobin levels by administration of Iron or blood transfusion. However, it is based on the underlying systemic disease. Newer trend of administrating erythropoietin stimulating agents are available.

Gunter Weiss *et al.*, 2005

Treatment	Anemia of chronic disease	Anemia of chronic disease with iron deficiency
Treatment of underlying disease	Yes	Yes
Transfusion	Yes	Yes
Iron supplements	No	Yes
Erythropoietic agent	Yes	Yes (patients who do not respond to iron therapy)

The other common therapies includes oral iron supplements that are easily available and of low cost but their effectiveness is questionable because of Hepcidin mediated iron block in the intestine that hinders absorption of iron, hence intravenous therapy can be

indicated. It is well known that IL-6 promotes the Hepcidin production, hence administration of IL-6 inhibitors like anti IL-6R antibody therapy have known to inhibit Hepcidin levels, decrease C-reactive protein levels within 1 week and also improvement in the hematological parameters over a period of 4 weeks after therapy was seen. But the administration of these newer agents such as cytokine inhibitors, anti-tumour necrosis factor are still under research and are not actively found to be prescribed in majority of population.

Ryan *et al.*,[18] Red blood cell transfusions are found to be beneficial in patients with more severe anemia and are appeared to be harmful in patients with mild to moderate anemia. Transfusion in patients with anemia was found to be associated with increased risk and mortality. Thus, anemia of chronic disease when untreated is harmful but the available intervention that increases the level of hemoglobin confers even greater harm. So it is better to treat the underlying cause of the disease than to treat the disease itself. If the primary cause is been eliminated a better improvement from the disease will be achieved. In case of ACD, treatment of underlying chronic disease should be the first attempt to be carried out in order to manage such complex diseases.

PERIODONTITIS - AN INFLAMMATORY DISEASE:[19]

Periodontitis is an inflammatory disease of the tooth supporting structures. It is broadly classified into 2 types Chronic and Aggressive Periodontitis. Chronic Periodontitis is commonly seen in adults whereas Aggressive Periodontitis is usually seen in young otherwise systemically healthy individuals. The characteristic feature of aggressive periodontitis is its rapidly progressive nature.

Periodontal infection not only causes a loss of tooth support, it is also considered as a risk factor for systemic conditions that includes rheumatoid arthritis, respiratory disorders, cardiovascular diseases and diabetes mellitus. A low grade inflammatory burden was suspected as a possible mechanism that has linking relationship between periodontitis and systemic disease. Infected and inflamed periodontal tissues act as a source of periodontal pathogens, virulence factors and inflammatory mediators that spread into circulation, creating and sustaining a chronic systemic inflammatory burden. Low grade inflammation causing slight elevation of the levels of inflammatory markers is recognised as an important mechanism in the pathogenesis of systemic diseases including metabolic syndrome, insulin resistance and Cardio vascular diseases (i.e atherosclerosis, acute myocardial infarction and stroke).

Tumor necrosis factor – alpha (TNF-α) is a pro-inflammatory mediator that initiates the production of collagenases, prostaglandins,

chemo and cytokines, cellular adhesion molecules and factors related to bone resorption. IL-17 is a pro-inflammatory cytokine that is produced by cluster of differentiation 4+ (CD4+) T-cell subset termed T-helper (Th) 17, that rapidly promotes recruitment of neutrophils, and also stimulates other cells to produce pro-inflammatory mediators such as IL-1β and -6, TNF-α and an acute phase reactant C-reactive protein (CRP) that is related to vascular inflammation. Elevated levels of circulating TNF-α, IL-6 and -17 and CRP have been related to an increased risk for cardiovascular events and other systemic diseases.

Severe periodontitis has been associated with increased serum levels of TNF-α, IL-6, IL-1β and CRP, thus a role for periodontal diseases as a potential risk that contributes to general inflammation and development of systemic disease was suggested. Interventional studies also hypothesized that treating periodontal disease could result in decreasing the circulating levels of pro-inflammatory mediators.

Duarte *et al.,* conducted a pilot study and evaluated the serum levels of TNF-α, INF-γ; and IL-4, -17 and -23 before and after phase I periodontal therapy in Aggressive periodontitis, chronic periodontitis and control group. Subjects with GAgP had a higher circulating levels of TNF-α, IL1 than subjects with chronic periodontitis and healthy controls, although clinical periodontal condition and serum levels of cytokines were improved significantly as a result of periodontal therapy.

Periodontium can be stated as a cytokine reservoir, the pro-inflammatory cytokines TNF-α, IL-1β, and gamma Interferon as well as Prostaglandin E_2 (PGE_2) reach the tissue in higher concentration in periodontitis condition.[7]The subgingival microbiota in periodontitis patients provides a persistent Gram-negative bacterial challenge to the host that would be met by a potent immunomodulatory response. The endotoxins and LPS released by these organisms have a ready access to tissues and to the circulation via sulcular epithelium that is frequently ulcerated. By this given mechanism the periodontal infection can have an impact on systemic health, the diseases and conditions possibly influenced by periodontal infection are cardiovascular diseases, diabetes, adverse pregnancy outcomes, and respiratory diseases. One of the major mechanism by which Anemia of Chronic Disease (ACD) develops is the increased production of cytokines. The increased production of cytokines tends to depress erythropoietin production leading to development of anemia. **Lainson *et al.,*** and **Chawla *et al.,*** suggested Anemia as a cause or pathogenesis of periodontal diseases.

Later in **1935 Epstein** first put forward the concept that Anemia is secondary to periodontitis [20].

William's RC, Offenbacher S *et al.,*2000 described periodontal medicine as a rapidly emerging branch of Periodontology focussing on establishing a strong relationship between periodontal disease and

systemic health and offers new insights of the oral cavity as one system interconnecting with the whole human body.

Studies in the past done to clarify association between periodontal disease and decreased red cell parameters were either cross-sectional or longitudinal and described the co-existence of chronic periodontitis and anemia. This is the first interventional study carried out to discover the effect of SRP in the improvement of Hematological parameter in **Generalized Aggressive Periodontitis** patients.

REVIEW OF LITERATURE

Cash JM *et al.*, 1989 [21]

Conducted a prospective study and hypothesized that many patients have anemia with the characteristics of ACD (Anemia of Chronic Periodontitis) but do not have any of the infections, inflammatory or neoplastic disorders usually associated with ACD, therefore evaluated a series of anaemic patients admitted to county hospital. 7 patients with iron deficiency were not considered, 90 with ACD were compared with 75 non-ACD patients. Anemia in ACD patients was found to be severe, the mean hematocrit value was 31% and 20% of patients had hematocrit values below 25%. The serum iron-binding capacity was quite lower in ACD and found to be normal in non-ACD patients. This study pointed out the nosological and terminological problems in ACD and stated that anemia may occur in infectious, inflammatory and neoplastic diseases for a variety of other reasons such as bleeding, hemolysis or marrow involvement and also included that patients might have anemia and the abnormalities of iron distribution characteristic of ACD but lack one of the traditional "chronic diseases". The abnormality in the distribution of iron may not be present in patient with iron deficiency, thus it was anticipated that further insight into mechanisms would lead to a better definition of ACD and concluded that until a proper etiologic and pathogenetic mechanisms were understood, a broad inclusive view about ACD seem to be prudent.

Greenstein G 1992 [22]

Presented a literature review in order to clarify the potential for successful treatment of patients with periodontitis using mechanical non-surgical therapy. Clinical, microbiological and histologic responses to phase I therapy were evaluated to provide guidelines for expected treatment outcomes. Clinical trials were demonstrated that non-surgical therapy was sufficient enough to resolve inflammation and arrest periodontitis and concluded that most patients with mild to moderate periodontitis can be successfully treated with non-surgical therapy.

Loos G *et al.*, 2000 [23]

The purpose of this study was to investigate systemic levels of inflammatory markers of cardiovascular diseases in patients with destructive periodontal disease in comparison with periodontally healthy individuals. Plasma C- reactive proteins and IL-6 were measured particularly and total leukocyte and differential leukocyte counts measurements were also performed. CRP levels and IL-6 levels were higher in generalized periodontitis and localized periodontitis when compared to healthy controls. Leukocytes were also elevated in generalized periodontitis compared to localized periodontitis and controls. Result of this study concluded that periodontitis results in higher systemic levels of CRP, IL-6 and neutrophils.

Hutter JW *et al.*, 2001 [5]

Aimed to evaluate the signs of Anemia in patients with Periodontitis. 39 patients with severe periodontitis, 71 with moderate periodontitis and 42 healthy controls were participated in this study. Several hematological parameters were determined from peripheral blood samples. The results indicated that periodontitis patients had a lower hematocrit, hemoglobin and erythrocyte counts but higher ESR value proving that periodontitis patients may have many systemic problems.

Christan C *et al.*,2002 [24]

The purpose of the study was to examine the systemic effect of non-surgical therapy on white blood cell count and differential blood count in smoking and non-smoking generalized aggressive periodontitis patients. Total number of 27 adult patients with previously untreated generalised aggressive periodontitis were recruited of which 13 smokers and 14 non-smokers were segregated. Periodontal examinations were performed at baseline and at the end of third month after subgingival therapy. Periodontal parameters like pocket Probing depth and Relative attachment level were recorded. Venous blood samples were taken to analyse WBC counts and differential blood counts. Clinical and demographic differences were not found between smokers and non-smokers, following non-surgical therapy a reduction in the WBC, neutrophil and platelet counts were seen in non-smokers while smokers' platelet counts were only reduced. Non-smokers had a

significant higher reduction of WBC counts and neutrophils than smokers. Thus, study concluded that a therapeutical intervention might have a systemic effect on the blood count in generalized aggressive periodontitis patients.

Loos G *et al.*, 2005 [25]

Reviewed and summarized the knowledge on systemic levels of systemic markers of inflammation in periodontitis. From peripheral blood sample cellular factors viz., WBCs, RBCs, and thrombocytes were discussed, furthermore plasma levels of acute-phase proteins, cytokines and coagulation factors were reviewed. From the available literature the information reviewed were total Number of leukocytes and plasma CRP levels and the levels were higher in patients with periodontitis than controls. The erythrocyte and haemoglobin levels were lower in periodontitis patients and stated that it tends towards ACD. The systemic markers of inflammation discussed in the review were also regarded as markers for cardiovascular disease.

Havemose – Poulsen *et al.*, 2006 [26]

Aimed to elucidate whether patients with Localized Aggressive Periodontitis (LAP), Generalized Aggressive Periodontitis (GAP) and Juvenile Idiopathic Arthritis (JIA) and Rheumatoid Arthritis(RA) share clinical and blood parameters distinguishing them from subjects free of diseases. All subjects underwent a standardized interview, blood sampling and an intraoral examination, clinical parameter like plaque

index, bleeding on probing, probing depth, clinical attachment level and alveolar bone loss on radiograph were recorded. Hematological parameters like erythrocyte fraction, leukocyte and differential counts, ESR, CRP, immunoglobulin IgM and IgA rheumatoid factors (RFs) and antibodies to cyclic citrulinated peptides were included. The findings concluded that young adults with RA might develop periodontal destruction and these patients require attention. GAP patients may present with elevated levels of traditional markers of inflammation similar to patients with RA and JIA. Thus, similarities in periodontal and blood variables were found in individuals with GAgP, JIP and RA, distinguishing them from controls.

Erdemir *et al.*, 2008 [27]

Investigated the effect of cigarette smoking on clinical parameters and signs of ACD in Chronic Periodontitis (CP) patients. 88 patients with CP including 45 smokers with age group of 30-69 years and 43 non-smoking volunteers with age range of 32-61 years were enrolled in the study. The findings resulted in higher PI, PD and CAL score in smokers than non-smokers ($p<.05$) and number of erythrocytes and levels of hemoglobin, Hematocrit and iron were found to be lower in smokers compared to non-smokers. Thus, concluded that cigarette smoking may be effective in the signs of anemia of chronic disease in patients with chronic periodontitis.

Shi D *et al.*, 2008 [28]

The study aimed to explore the characteristics of peripheral blood cellular and serum protein parameters in individuals with Aggressive periodontitis. 150 aggressive periodontitis and 94 healthy control were recruited, clinical parameters viz., PD, CAL and blood variables such as leukocyte, neutrophil and lymphocyte counts as well as serum protein including total protein, albumin, globulin, albumin/globulin ratio were analysed. The results indicated elevated peripheral leukocyte numbers and serum globulin levels and decreased serum albumin and albumin/globlin ratios compared to controls.

Agarwal N *et al.*, 2009 [4]

Carried out an interventional study to evaluate Periodontitis as one of the etiological factors leading to ACD. 30 chronic generalized periodontitis male patients with Hb below 15mg/dl and serum ferritin above 30ng/ml were selected, various blood parameters (RBC count, ESR, MCV, MCH & MCHC) and periodontal parameters (PI, GI, PD & CAL) were recorded at baseline and after periodontal therapy same parameters were recorded at the end of 3rd month and 1 year. The result showed that correction of periodontal inflammation lead to a significant increase in haemoglobin concentration and erythrocyte counts. The ESR value was decreased indicating resolution of inflammation and lesser improvement was found with the blood indices. Thus, evidence concluded that treatment of periodontitis leads to an improvement in hematocrit and other related blood parameters in

chronic generalized periodontitis patients with anaemia, suggesting that periodontitis like other chronic diseases might cause anaemia.

Alijohani A *et al.*, 2009 [29]

Aimed to investigate the association between hemoglobin level and the severity of chronic periodontitis. Data collection from 124 systemically healthy chronic periodontitis patients were done. Periodontal parameters like bleeding on probing, probing depth, clinical attachment loss, mobility, missing teeth were recorded. Blood samples were collected for assessing hemoglobin concentration. The result of the study suggested that there were no significant correlation found between hemoglobin and the means of clinical attachment loss and bleeding on probing and also stated that there was no association between hemoglobin levels and periodontal status.

Duarte PM *et al.*, 2010 [19]

This pilot study aimed to evaluate the serum levels of tumour necrosis factor- alpha (TNF-α) and interleukin IL-4, 17 and 23 in subjects with generalized chronic periodontitis (CGP) and generalized aggressive periodontitis (GAgP) before and after non-surgical periodontal therapy. The results after periodontal therapy in both group demonstrated a significant improvement in clinical periodontal status. At baseline, the concentration of TNF-α and IL-17 were higher in GAgP group compared to the other groups and a significant decrease in serum concentration of TNF-α and IL-17 after therapy in GAgP was

seen. It has been concluded that GAgP group has a higher levels of TNF-α and IL-17 than subjects with chronic generalized periodontitis and controls.

Gokhale SR *et al.*, 2010 [30]

Aimed to probe the association between chronic periodontitis and anaemia. Evaluated the peripheral blood samples for red blood cell parameters and compared it in male patients with chronic periodontitis patients and healthy controls. A total number of 60 systemically healthy male patients with mean age group of 38 years were enrolled of which 30 healthy controls and 30 severe periodontitis patients were assessed. The result showed that patients suffering from CP had a lower number of RBCs and Hb than controls, thus it was concluded that like other chronic condition, CP lead to anemia.

Shin-Yu Lu *et al.*, 2010 [31]

Presented a case report of severe anemia caused by severe periodontitis in a 50 years old female. Patient had a complaint of general weakness and looked debilitated. Hematological parameters revealed increased WBCs and decreased RBCs. Dental condition was poor and diagnosed as severe periodontitis. After dental treatment (extraction of hopeless teeth, oral prophylaxis and delivering new dentures) anemic state was reversed to health and improved the quality of life.

Naik V *et al.*, 2010 [32]

Conducted a case-control study to evaluate the levels of systemic haematological markers indicative of anemia in patients with generalized severe chronic periodontitis. 30 systemically healthy urban male patients divided into 2 groups based on periodontal status as group A (n=15) with generalized, severe chronic periodontitis and group B patients with clinically healthy periodontium as control group (n=15). Blood samples were collected for investigating hemoglobin, total number of red blood cells, hematocrit/PCV, ESR and blood indices. The results concluded that the mean values of hemoglobin, RBCs, PCV and ESR were higher in group A than group B indicating link between severe periodontal disease and anemic status.

Pradeep AR *et al.*, 2011 [33]

Investigated whether chronic periodontitis patients have anemic status and analysed the effect of phase I therapy after 6 months. A total of 187 chronic generalized periodontitis subjects were made to participate, 60 individuals with reduced red cell parameters entered into second part of the study in which they were treated with non-surgical therapy, clinical and blood parameters were repeated at 3 and 6 month. The results suggested that statistical improvements were seen after phase I therapy over a six month period. Thus, it strengthens the hypothesis that chronic periodontitis may lead to anemia and provided evidence that non-surgical periodontal therapy could improve the anaemic status in individuals with chronic periodontitis.

Yamamoto T *et al.*, 2011 [34]

The study utilized longitudinal data from health checkups to clarify the relationship between periodontal disease progression and changes in the erythrocyte parameter in 120 subjects (35 men and 85 women from Japanese population). Comprehensive screening and dental checkups were done, progression group comprised 30 subjects and had observed changes in the erythrocyte counts compared with non-progression group. Hence, suggested that progression of periodontal disease is associated with a decrease in erythrocyte count in rural Japanese population.

Garg B *et al.*, 2012 [35]

Undertaken this study to assess routine hematological parameters and body iron stores of blood donors to identify whether they were potentially prone to develop iron deficiency anemia in 116 male donors, Pre-donation Hb, Hemogram and serum ferritin investigations were done and allocated into 2 groups based on the number of donations. Findings suggested that first time donors had higher mean serum ferritin than repeated donors, recommending iron supplements for adequate period post donation.

Malhotra R *et al.*, 2012 [8]

Evaluated the effect of chronic periodontal disease on erythrocyte count, Hemoglobulin and Hematocrit(HCT) and assessed the changes produced in these parameters after the provision of

periodontal therapy in 40 systemically healthy subjects in the age group of 25-50 years. Subjects were allocated into two groups as group A with chronic generalised gingivitis and group B with chronic generalised periodontitis on the basis of clinical findings. After performing complete oral prophylaxis patients were adviced to report after 3 weeks and 3 months, blood and clinical parameters were re-evaluated to analyse the difference after phase I therapy. The obtained results showed that the mean values of RBC count, Hb, and HCT were lower in group B than group A and a significant increase at the end of 3 months in group B was observed.

Virdi HK *et al.*, 2013 [36]

Investigated the association between hematological parameters and the severity of chronic periodontitis in a clinical trial that involved 80 systemically healthy individuals and divided into 2 groups based on gingival findings 40 subjects (20 male and 20 female) with moderate to severe chronic periodontitis as test groups and other 40 subjects with clinically healthy gingiva as controls. The results indicated lower levels of Hb and RBC while the ESR value seemed to be higher in test group than control hence a positive relationship was observed between blood parameters and severity of chronic periodontal disease.

Liu J *et al.*, 2013 [37]

Evaluated the changes in the clinical parameters and the prevalence and quantities of 3 major Periodontopathic organisms

namely *P.gingivalis, A.actinomycetemcomitans* and *T. Forsythia* in subgingival plaque of patients with generalised chronic periodontitis and generalised aggressive periodontitis in response to Phase I therapy. The findings concluded that non-surgical periodontal therapy found to be effective in the treatment of clinical symptoms and the 3 major periodontal pathogens were controlled after therapy in both groups.

Kalburgi NB *et al.*, 2013 [38]

Investigated the clinical parameters and signs of Anemia of Chronic disease in chronic periodontitis patients in non-tobacco users, smokers and smokeless tobacco users. 90 chronic periodontitis patients of which 30 non-tobacco users, 30 smokers and 30 smokeless tobacco users in the age group of 36-59 years were included in the study. The haemoglobin concentration, total red blood cell count and Hematocrit value were analysed and found to be lower significantly in smokeless tobacco users group followed by smokers group and then by non-tobacco user group. Thus, study indicated that periodontitis should be considered as chronic disease and together with the effect of cigarette smoking and / or smokeless tobacco might affect the systemic markers related to Anemia of chronic disease.

Nair SK *et al.*,2013 [39]

The main objective of the study was to investigate the relationship between anemia and periodontal disease. A total number of 60 subjects were taken from both the genders with a mean age of 46

years and they were comprised into 3 groups as group I healthy subjects, group II gingivitis and group III with periodontitis (n=20 in each group). Periodontal parameters and orthopantamographs were taken for all groups along with 5 ml of venous blood samples collected for hematological assessment. Comparison of hematocrit values (Hb%, MCHC%) in periodontitis, gingivitis and healthy groups resulted in lower PCV value in periodontitis than with gingivitis and healthy individuals. Thus, the study provided evidence that like other chronic infections periodontitis tend towards anemia.

Jenebian N *et al.*, 2013 [40]

Conducted a case-control study to investigate the correlation between haematological parameters associated with anemia and moderate chronic periodontitis. 60 systemically healthy male were enrolled and blood samples were collected of which 30 men were diagnosed with moderate chronic periodontitis. Hemaological and clinical parameters were assessed. A reduction of MCV, MCH, Hb, Hematocrit value, Serum iron (SI) and total iron binding capacity (TIBC) factors were observed with increasing gingival index (GI), clinical attachment level (CAL) and bleeding on probing (BOP). Thus, it was concluded that correlation was observed between some haematological parameters associated with anemia and moderate chronic periodontitis.

Anand PS *et al.*, 2014 [1]

Conducted a case-control study to determine whether generalised aggressive periodontitis (GAP) is associated with reduced erythrocyte counts and reduced hemogloin levels, in 64 patients with GAP and compared with 58 periodontally healthy controls. Hematological parameters such as erythrocyte counts, hemoglobin concentration, hematocrit and erythrocyte indices and periodontal parameters such as (PI, GI, CAL, PD) were recorded. The findings suggested that patients with GAP tend to have lower RBC counts and Hb concentration when compared to controls.

Patel MD *et al.*, 2014 [20]

The purpose of the randomized controlled double blind study was to investigate chronic periodontitis patients for anemic status and analyse the effect of non-surgical therapy on red blood cell parameters over a period of 6 month. 100 systemically healthy male patients were recruited of which 50 were periodontally healthy and 50 had chronic generalised periodontitis. Clinical and hematological parameters were assessed at baseline and at 6 month after phase I therapy. The results suggested that like other chronic condition periodontitis could lead to ACD and non-surgical therapy improved the anaemic status in test group.

Kolte RA *et al.*,2014 [41]

Aimed to assess and compare the various blood parameters, total leukocyte count (TLC), Bleeding time (BT) and clotting time (CT) in healthy and chronic periodontitis subjects. The results stated that periodontitis group showed lower RBC level, MCHC concentration and increased TLC compared to healthy individuals and concluded that periodontitis might tend toward anemia and a marked leukocytosis due to increased circulating neutrophils and lymphocytes were seen.

Shetty MK *et al.*,2014 [42]

Conducted a case control study to assess the effect of nonsurgical periodontal therapy on hemoglobin levels in chronic periodontitis patients. 40 anemic subjects with reduced hemoglobin levels (<8 g/dl), 20 were periodontally healthy and 20 with periodontitis were involved. Blood samples were collected before and after non-surgical phase, the study results showed that there was an improvement in hemoglobin level in test group after therapy hence it was concluded that phase I periodontal treatment helped in the improvement of anemic status in periodontitis patients.

Kundu D *et al.*, 2014 [43]

Undertaken this study to assess the relation between expression of ABO blood group and HLA antigen, defective PMN adhesion & β2 integrin expression and percentage of CD14 + CD16+ monocytes in CP and AgP patients compared with healthy subjects. The study results

summarized that ABO blood groups and HLA phenotypes with periodontal diseases cannot be established. Leukocytic functional defects were found in aggressive periodontitis and a statistically significant percentage of CD14+ CD16 and CD45RA monocytes were found in aggressive periodontitis subjects when compared with normal control and chronic periodontitis patients.

Chakraborthy S *et al.*, 2014 [44]

Aimed to investigate the differences in concentrations of serum ferritin in patients with and without periodontal disease before and after non-surgical periodontal therapy and correlated these values with clinical variables related with periodontal disease. These findings concluded that increased level of serum ferritin in chronic periodontitis were found than in controls and post treatment results showed reduced serum ferritin levels comparable to normal range or equal to control subjects.

Mishra P *et al.*, 2014 [45]

Aimed to find the relationship between erythrocyte parameters and chronic periodontitis. A total number of 50 patients were chosen for study and all had atleast 30% or more of the teeth with > 4mm pocket probing depth and other clinical and hemotological variables were determined. The mean values of hemoglobin and red blood cell indices were found to be significantly lower, while the value of ESR was higher in test group compared to control group the result suggested

mild anemia. Thus, the study concluded that a positive relationship was observed between the hemotological parameters and the severity of periodontal disease.

Kaur N *et al.*, 2015 [46]

Reviewed to provide an overview of causes, signs and symptoms of different types of anemias with emphasis on the oral manifestations and their influence on the treatment plan of dental health professionals. This review concluded that dental health professional might be the first to recognize the presence of anaemia, thus their role in the diagnosis and management of the various types of anemia cannot be underestimated.

Joshipura V*et al.*, 2015 [47]

The purpose of the review was to highlight the causes and therapeutic concepts of aggressive periodontitis which was rapidly progressive in nature. Authors reviewed not only about the clinical, microbiological, immunological and genetic aspects of pathogenesis of aggressive periodontitis but also the diagnostic criteria of the disease and appropriate non-surgical and surgical treatment options. The conclusion stated that aggressive periodontitis affects smaller percentage of population that seemed to be influenced mainly by specific microorganism, host response and genetic predisposition as it is aggressive in nature it requires early diagnosis and treatment to prevent further loss of periodontium.

Santhosh HN *et al.*, 2015 [48]

Conducted an observational study to estimate various hematologic parameters suggestive of Anemia of Chronic disease in patients with chronic periodontitis. 40 patients were selected and categorized into cases and controls based on the presence and absence of chronic periodontitis. Complete hemogram, hematocrit, ESR and estimation of serum ferritin levels were done. The results showed significant difference in number of neutrophils, ESR, RBC and serum ferritin value in subjects with severe generalised chronic periodontitis compared to periodontally healthy patients. Thus, it was concluded that chronic generalised periodontitis by means of an inflammatory process might lead to ACD.

Khan NS *et al.*, 2015 [49]

Conducted a case-control study to assess the red blood cell parameters for signs of anemia in patients with mild, moderate and severe chronic periodontitis. 80 healthy male patients were divided into four groups based on periodontal findings as group I controls (clinically healthy periodontium), group II with mild periodontitis, group III with moderate chronic periodontitis and group IV with severe chronic periodontitis. Laboratory blood investigations viz total RBC, Hb concentration, PCV, MCV, MCH, MCHC were analysed. The obtained result showed a statistically significant decrease in RBC with increase grades of periodontitis. Thus the substantial decrease in RBC

with increase in severity of periodontal destruction concluded that anemia is linked with periodontitis.

Mupalla C *et al.*, 2016 [6]

Evaluated and compared the systemic markers related to anemia in peripheral blood of patients with chronic generalised severe periodontitis and healthy controls. 60 systemically healthy males were divided into 2 groups based on their periodontal status as group A (controls) healthy gingiva and group B (test group) chronic generalized severe periodontitis. Periodontal and hematological parameters were recorded in both the groups. The investigation resulted in reduced RBC, Hb, and PCV values in group B when compared to group A indicating that severe periodontitis has a definite systemic effects.

Anumolu H *et al.*, 2016 [50]

Evaluated the relationship between Anemia and Periodontitis by estimating blood parameters. Clinical and hematological parameters were evaluated in 50 healthy, 50 chronic generalized periodontitis patients. The results revealed a decrease in Hb and RBC values and increase in WBC counts in periodontitis group than in healthy and gingivitis group. And it was concluded that after periodontal therapy there was an improvement in Hematocrit and other blood parameters in periodontitis patients with anemia.

MATERIALS AND METHODS

An interventional study was conducted at the Department of Periodontology, Adhiparasakthi Dental College and Hospital, Melmaruvathur. A total number of 30 subjects were enrolled in the study with the age range of 18-40 years from the outpatient division of APDCH. Ethical clearance for the study was obtained from Institutional Review board (Reference No.2015-MD-Br II- VID-05/ APDCH).

All subjects participating in the study were informed about the nature of the study and all individuals signed in the written informed consent form. Clinical and blood parameters were measured at baseline, out of 30 patients, 17 patients met the inclusion criteria (serum ferritin above 30ng / dl) and these 17 patients were allocated for Non- surgical periodontal therapy (NSPT), two patients failed to report for NSPT. So, total 15 patients were considered for further investigation.

METHOD:

Group A: 15 subjects (Generalised Aggressive Periodontitis, before phase I therapy)

Group B: 15 subjects (Generalised Aggressive Periodontitis, after phase I therapy)

INCLUSION CRITERIA:

- Patients in the age group of 18-40 years
- Probing depth and clinical attachment level of ≥5mm
- Individuals were included in the study if they had a probing depth and a clinical attachment level of ≥ 5 mm on at least eight permanent teeth, of which at least three were not permanent first molars or incisors. (Classification of Periodontal Diseases and Conditions in 1999)
- Serum ferritin >30ng/dl.

EXCLUSION CRITERIA:

- Pregnant women.
- Lactating women.
- Individuals with history of any systemic disease.
- History of any antibiotic therapy or under periodontal treatment during 12 month period before the examination.
- Smokers.
- Iron deficiency anemia.

Following clinical index and periodontal parameters were recorded before and after Phase I therapy:

- Plaque index (Silness and Loe, 1964)
- Bleeding index (Ainamo and Bay)
- Probing pocket depth
- Clinical attachment level.

Following Red Blood cell parameters were analysed before and after phase I therapy:

- Haemoglobin concentration
- Total Red blood cell counts (RBC)
- Packed cell volume (PCV)/Haematocrit (HCT)
- Erythrocyte sedimentation rate (ESR)
- Blood indices (MCV, MCH,MCHC)
- Serum ferritin

METHODS TO BE FOLLOWED:

Consecutively, patients diagnosed with generalized aggressive periodontitis who reported to the Department of Periodontics and expressed willingness to participate in the study were recruited. Before undergoing the examination, a written informed consent was obtained from all the prospective study participants and they were subjected to the measurement of clinical index that includes plaque index and gingival bleeding index and clinical parameters including probing pocket depth and clinical attachment level at baseline and after 3 month post operatively.

BLOOD SAMPLE COLLECTION AND STORAGE:

After skin preparation, venous blood samples were obtained by vene puncture from the ante-cubital fossa under aseptic condition. After placement of a tourniquet, 5 ml of blood was collected using a disposable syringe from the median cubital vein in the antecubital

fossa. The 2.5 ml of collected blood sample was transferred into a plain vaccutainer tube for estimation of serum Ferritin levels, and the remaining blood sample was equally transferred into EDTA containing vial and sodium citrate containing vial. The blood samples transferred into sodium citrate containing vial was assigned to determine ESR whilst the blood samples collected in EDTA vial was used for estimation of hematological variables like (Hb %, RBC count, PCVand Blood indices).

PLAQUE INDEX: (Silness and Löe 1964) [51]

Plaque index was described by Silness J. and Loe H. in 1964 and fully described by Loe H. in 1967.

SCORING CRITERIA FOR THE PLAQUE INDEX SYSTEM

0 = No plaque.

1 = A layer of plaque adhering to the free gingival margin and adjacent tooth surface. The plaque is not visible to naked eyes, can be recognised only by running the explorer around the tooth surface

2 = Moderately accumulated soft deposits within the pocket, margin of the gingiva and/or adjacent tooth surface that is seen by the naked eye.

3 = Abundance of soft matter seen within the gingival pocket and/or on the gingival margin and the adjacent tooth surface.

CALCULATION:

Plaque index for per tooth= sum of total score from 4 areas of the tooth and divided by 4.

Plaque index for an individual= total plaque indices for all teeth are added and divided by total No.of teeth examined.

INTERPRETATION:

SCORE 0:Excellent oral hygiene

SCORE0.1 to 0.9:Good oral hygiene

SCORE 1.0 to 1.9: Fair Oral Hygiene

SCORE 2.0 to 3.0: Poor oral hygiene

GINIGVAL BLEEDING INDEX:[52]

The Gingival Bleeding index (GBI) of **Ainamo and Bay** was developed as an easy and suitable method for the examiner to assess a patient's progress in plaque control. The presence or absence of gingival bleeding can be determined by using a periodontal probe and gently probing the gingival crevice. The appearance of bleeding within 10 seconds indicates a positive score, which can be expressed as a percentage of the total number of gingival margins examined.

PROBING POCKET DEPTH:[52]

Probing depth is a measurement of the depth of a sulcus or periodontal pocket. **Pocket depth** refers to the **distance from the**

gingival margin to the base of the clinical pocket. Mesial and distal pockets are measured from the buccal aspect and as close as possible to the contact points. Facial and oral pockets were measured at the midline of the roots. Buccal and lingual pockets of multi-rooted teeth were measured at the mesial roots in order to avoid the furcation areas. Probing involves "stepping" a calibrated periodontal probe (UNC-15) around the tooth and recording the deepest point at each of six surfaces of the tooth: distofacial, facial, mesiofacial, distolingual, lingual, and mesiolingual. A probe reading that falls between two calibrated marks on the probe should be rounded upward to the next highest millimetre. Out of 6 surfaces per tooth, the highest probing depth value is taken as the probing depth of that individual tooth.

CLINICAL ATTACHEMENT LEVEL:[52]

Level of attachment refers to the **distance between the base of the pocket and CEJ**. The loss of attachment was assessed on the same surfaces of the same teeth and with the same probe as used for measuring the pocket depth. Following CEJ recognition, the distance from the gingival margin to the CEJ was measured. When the CEJ was located apical to the gingival margin, the loss of attachment would be the difference between the previously recorded depth of the pocket (A) and the distance (B) from the gingival margin to the CEJ: (A –B = loss of attachment). In cases where the marginal gingiva had been subject to recession and the CEJ was exposed, the loss of attachment equalled the sum of the pocket depth and the distance from the gingival margin to

the CEJ: A + B = loss of attachment. Pocket depth or loss of attachment of 1 mm or less was recorded as 1 mm, measurements exceeding 1 mm, but less than 2mm, were recorded as 2 mm.

HEMATOLOGICAL VARIABLES :[53, 54, 55]

HEMOGLOBIN is the iron-containing oxygen-transport metalloprotein in the red blood cells. The average hemoglobin content in blood is **11 to 16.38 g/dl**. The value differs according to age and gender of an individual. At birth it is increased25 g/dl. In adult males it is 16 g/dl. In adult females it is 14 g/dl.

RED BLOOD CELL COUNT: RBCs or Erythrocytes are the non-nucleated formed elements in the blood. They are called as Red blood cells because of the presence of colouring pigment called hemoglobin. Normal value ranges from **4 to 5.5 millions /cu mm** of the blood. In adult males, it ranges 5.4millions / cu mm and in adult females it is 4.8millions / cu mm.

ERYTHROCYTE SEDIMENTATION RATE (ESR): It is the rate at which the erythrocytes settle down, if a column of anti-coagulated blood sample is allowed to stand on a vertical tube, the RBCs begin to settle down due to gravity with a supernatant layer of clear plasma. ESR tends to be increased in women with increasing in age and during pregnancy. When there is an increase in fibrinogen, immunoglobulins or acute phase reactants the RBCs cluster together to form *rouleux* and

settle down faster. It will be increased in infections, malignancies and chronic disease states like Rheumatoid arthritis. It will be decreased in polycythemia, hyperviscosity etc. Two different methods to determine ESR are Westergren method and Wintrobe method. Values for ESR vary in both the methods because of differences in the tube length and shape. It is not a diagnostic test but can be taken as a prognostic one that helps to monitor the progress of an inflammatory disease.

WESTERGREN METHOD: (Normal value)

Men it is up to 15 mm/hour

Women it is up to 20 mm / hour.

WINTROBE METHOD:

Males: 0-9 mm per hour

Females: 0-15 mm per hour.

HEMATOCRIT: (PACKED CELL VOLUME)

The hematocrit is the fraction of the blood that composed of red blood cells, as measured by centrifuging blood in a 'Hematocrit tube' until the cells become tightly packed in the bottom of the tube. The proportion of the blood that is Erythrocytes (RBC) is called the *Hematocrit*. If the Hematocrit is 40 then 40% of the blood volume is cells and the remainder is plasma. In severe anemia PCV may fall as low as 0.10. Conversely, in some conditions where there is excessive production of RBCs resulting in polycythemia, the haematocrit can rise to 65%.

NORMAL VALUE OF PCV:

Adult Men: 47 Adult Women: 42

BLOOD INDICES:

Blood indices are the calculations derived from RBC count, haemoglobin content of blood and PCV; it helps in diagnosis of the type of anemia.

MCV: It is the average volume of a single RBC and it is expressed in cubic microns. Normal MCV is **87 cu µ (77 to 93 cu µ)** if MCV is normal the RBC is called Normocytic, if cells with MCV is greater than 95 fL is known as Macrocytic and cells with MCVless than 82fL are called as Microcytes.

MCV =Hct*10/ RBC (10^6 /µl)

MCH: It is the quantity or amount of hemoglobin present in one RBC. It is expressed in Picograms. Normal value of MCH is **30 pg (27 to 32pg).**

MCH = Hb *10/RBC (10^6 /µl)

MCHC: Concentration of Hemoglobin in one RBC. It is the amount of hemoglobin expressed in relation to the volume of one RBC. It is expressed in percentage. Normal value is **34 (30 to 35 g/dl).** Normal MCHC then the RBC is Normochromic, cells with MCHC less than (<25 g/dl) are called as hypochromic. A single RBC cannot be

hyperchromic because the amount of hemoglobin cannot increase beyond normal.

MCHC = Hb*100/Hct

SERUM FERRITIN:

Ferritin is the most common type of iron storage in the human body. Ferritin molecules can be found in the cytoplasm of the reticuloendothelial system specifically in the liver and spleen. Iron is situated in the center of the molecule in the form of ferric hydroxyphosphate, this may contain as many as 4,500 iron atoms. The drop in serum ferritin levels can indicate an iron deficiency. The level of serum ferritin acts as an indicator of the quantities of iron in the human body.

PROCEDURE:

VIDAS® ferritin is an automated quantitative test for the determination of serum ferritin using the ELFA (Enzyme Linked Fluorescent Assay) technique.

PRINCIPLE:

The principle is based on one-step enzyme immunoassay sandwich method with a final fluorescent detection (ELFA). The Solid Phase Receptacle (SPR®) serves as the solid phase and as the pipetting device for the assay. The assay reagents are ready-to-use and are pre-dispensed in the sealed reagent strips. The steps of the assay are

performed by the machine automatically. The reaction medium is cycled in and out of the SPR several times. At the final detection step, the substrate (4-Methyl-umbelliferyl phosphate) is cycled in and out of the SPR. The conjugate enzyme catalyzes the hydrolysis of this substrate into a fluorescent product (4-methyl-umbelliferone) the fluorescence of which is measured at 450 nm. The intensity of the fluorescence is proportional to the concentration of antigens present in the sample. The final step of assay results are calculated by the instrument automatically in relation to the calibration curve that is stored in the memory, and printed results can be obtained.

PROCEDURE FOR ESR:

An ESR test is also called as SedRate test that measures the speed at which red blood cells settle at the bottom of an upright test tube. It is important because when the presence of abnormal proteins are found in the blood, mainly because of inflammation or infection, they cause red blood cells to clump together and sink faster. A disposable ESR pipette is used for performing WESTERGREN'S ESR determinations directly in a 12 * 75 mm blood collection tube or vaccutainer tubes.

Procedure is done using QUALES (single use ESR pipettes). Collect 1.6 ml of blood in 0.4 ml of sodium citrate 3.8% solution. Gently mix the blood, and insert the lower end of pipette bearing vaccum plug into the blood collection tube and using continuous force,

push the pipette down to the bottom of the blood collection tube. The blood will raise automatically into the pipette and stop at the zero mark. Place the assembly (Tube + Pipette) absolutely vertical on a suitable stand and allow the blood cells to sediment without disturbing it for 60 mins. After 60 minutes the numerical results can be read in millimetres directly from the imprinted scale visible on the pipette.

FULLY AUTOMATED HEMATOLOGY ANALYSER

The estimation of RBC count, Hemoglobin concentration, Packed cell volume (PCV) / Hematocrit (HCT), and Blood indices (MCV, MCH, MCHC) are done using Fully Automated Hematology Analyser (GB-325 Genuine Biosystem).

Basic Armamentarium:

1. Mouth mirror
2. UNC 15 probe
3. Tweezers
4. Universal curettes - 2R/2L & 4R/4L
5. Gracey curettes- #1-14
6. Surgical gloves
7. Mouth masks
8. Cotton rolls
9. EDTA containing vial, sodium citrate containing vial and plain vacutainer tube
10. 2 ml and 5 ml syringe

FIGURE 1: ARMAMENTARIUM

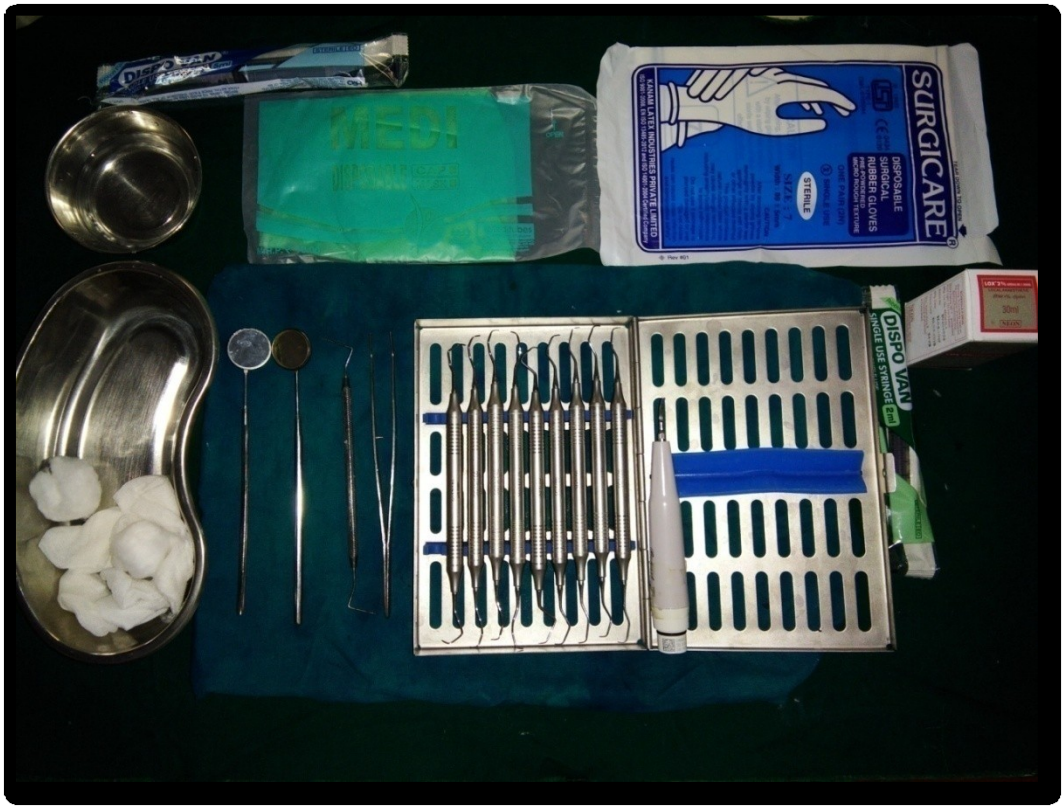


FIGURE 2 : PRE-OPERATIVE

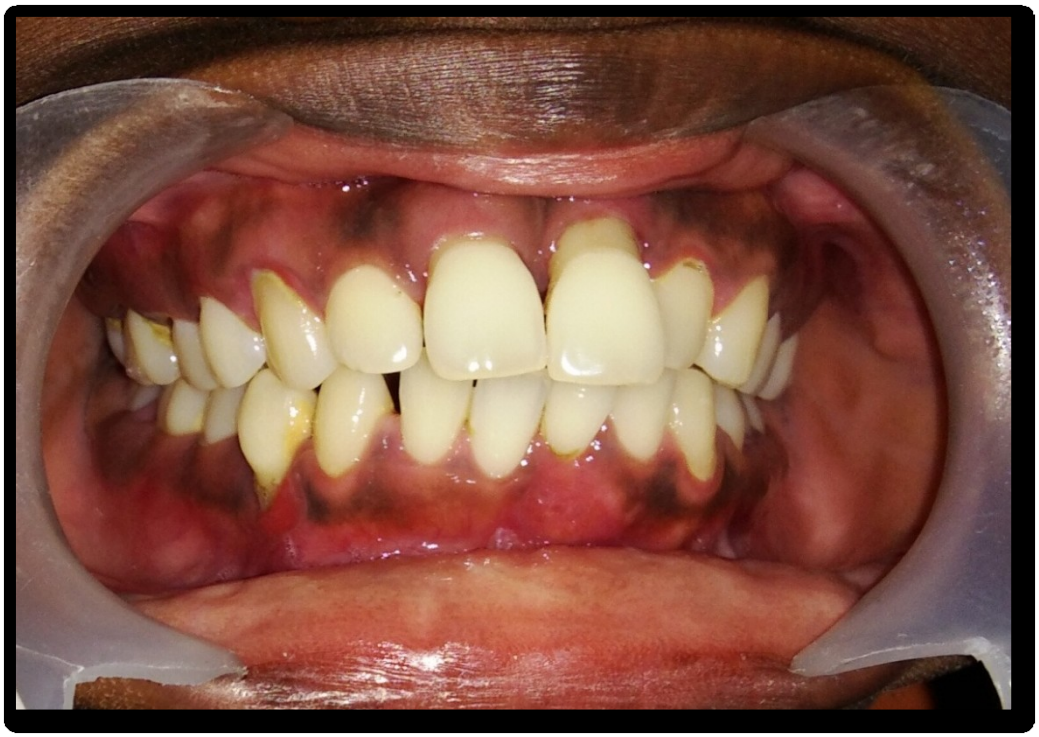


FIGURE 3 : PROBING DEPTH (BASELINE)

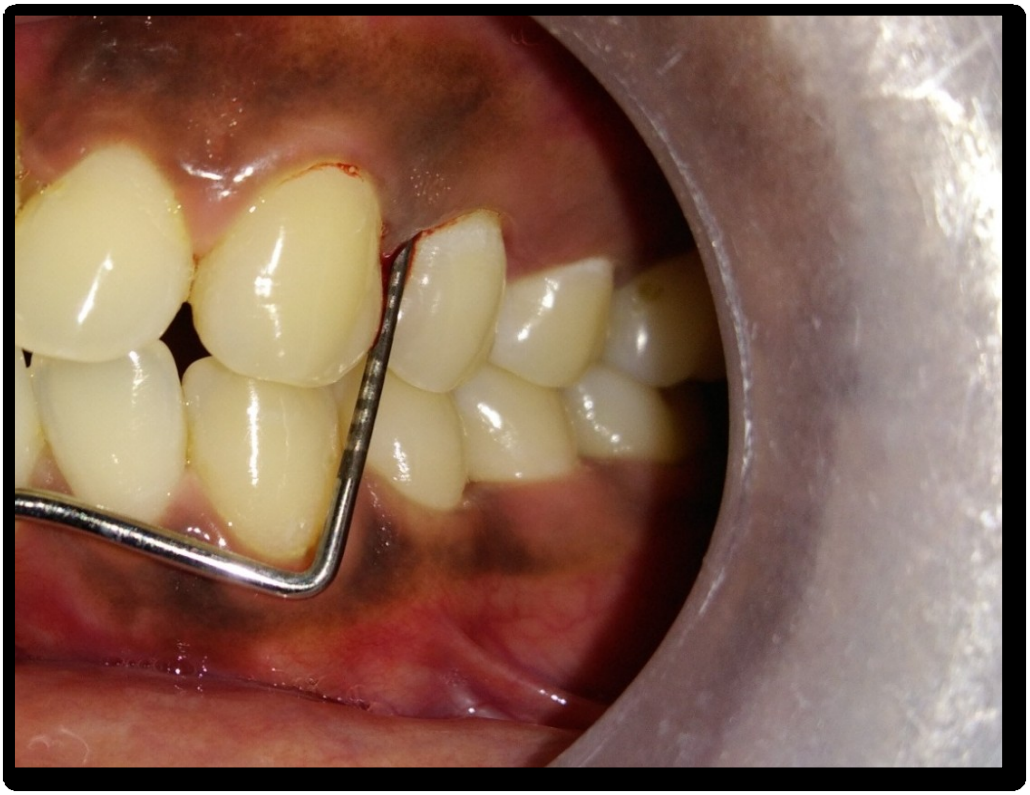


FIGURE 4 : ORTHOPANTAMOGRAPH (Pre-operative)

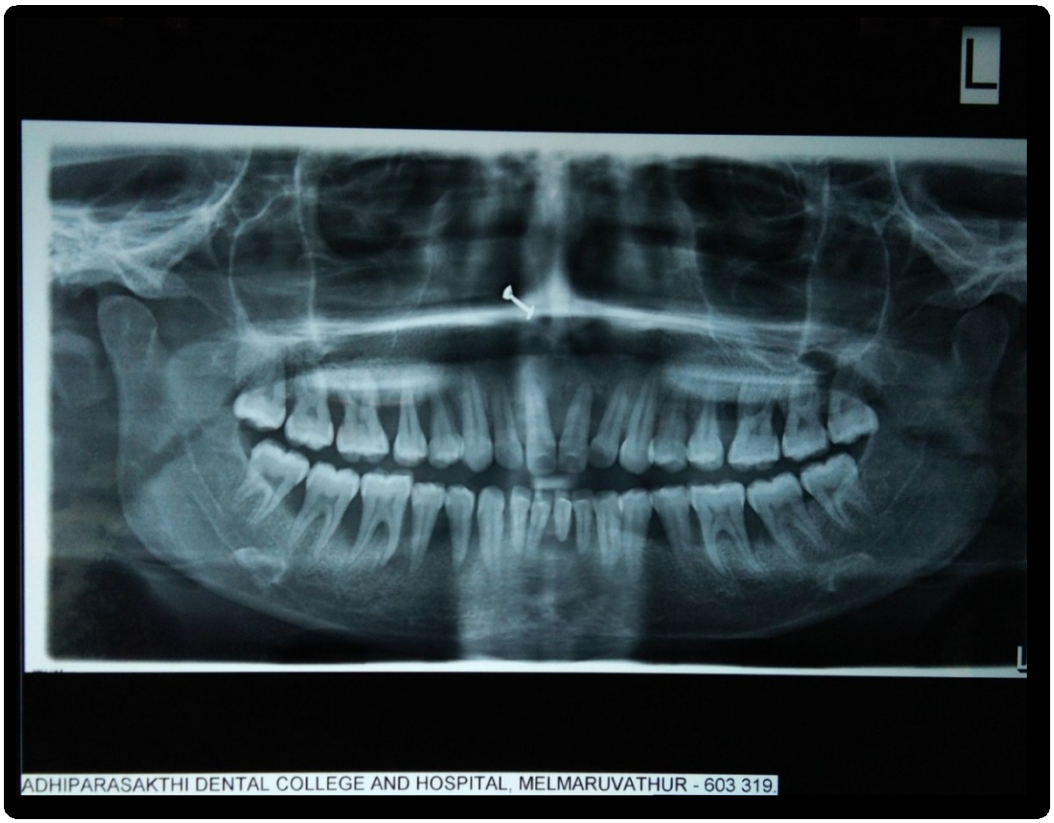


FIGURE 5 : POST-OPERATIVE

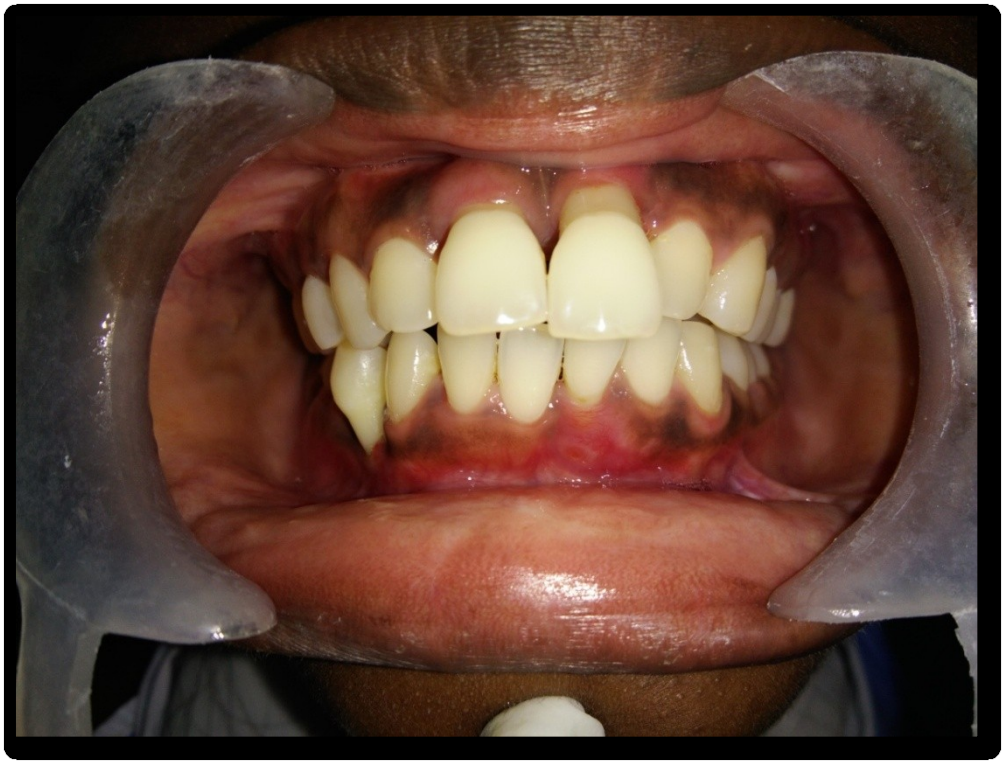


FIGURE 6 : PROBING DEPTH (3rd MONTH)

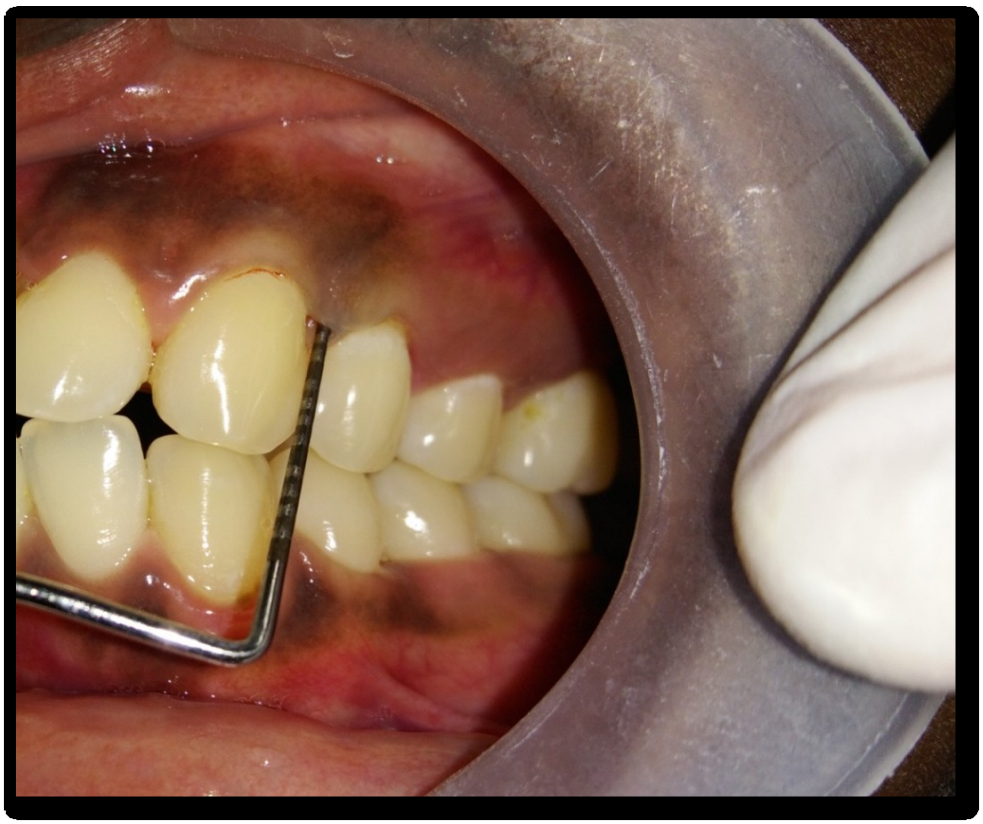


FIGURE 7 : COLLECTION OF BLOOD

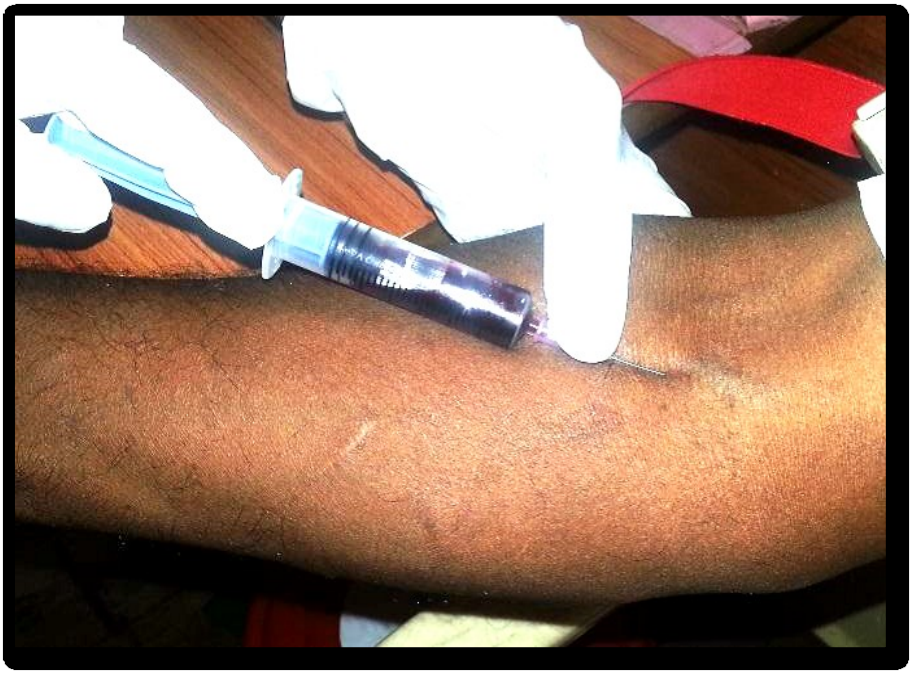


FIGURE 8 : COLLECTED BLOOD SAMPLES

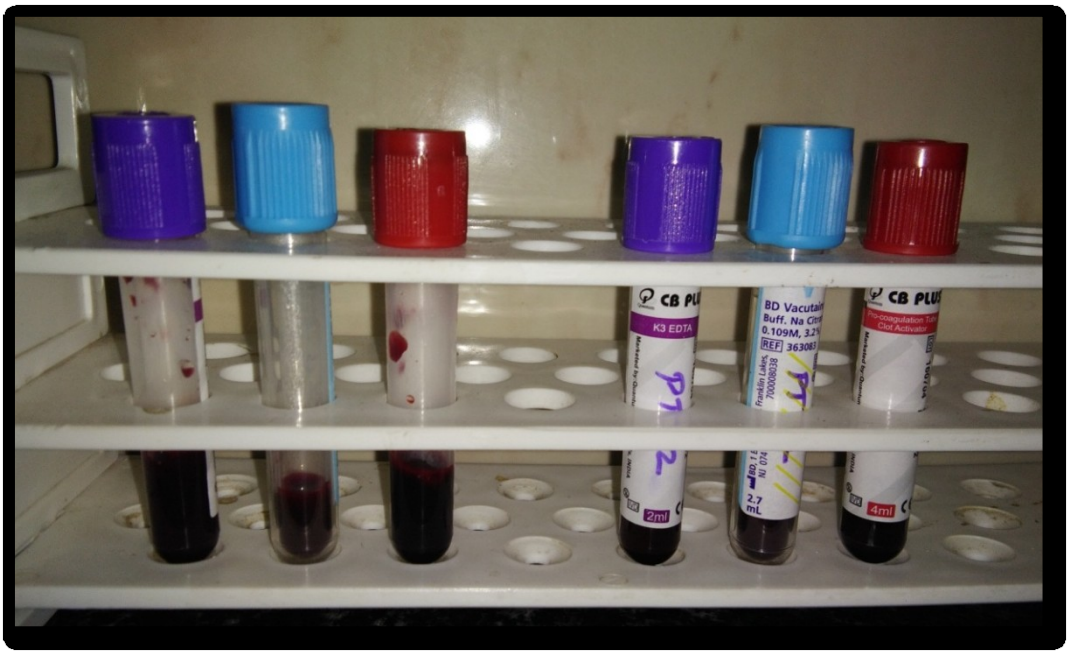


FIGURE 9 : FULLY AUTOMATED HEMATOLOGY ANALYSER

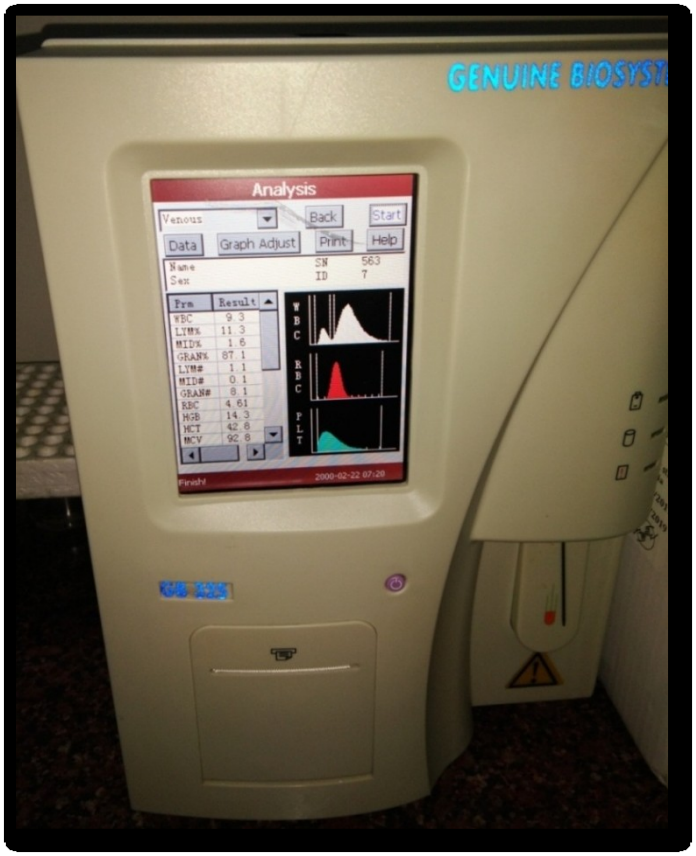


FIGURE 10 : CENTRIFUGED SERUM

Figure 11 : VIDAS ® FERRITIN

FIGURE 12 : ESR - WESTERGREN TUBE

RESULTS

The study was conducted to evaluate the effect of Non-surgical periodontal therapy on Red blood cell parameters of Generalized Aggressive Periodontitis patients. A total number of 30 patients diagnosed with Generalized Aggressive Periodontitis were selected from outpatient section of Department of Periodontics, Adhiparasakthi Dental College and Hospital, Melmaruvathur, Tamil Nadu. All clinical parameters were measured at baseline and blood samples were collected and sent for estimation of Red blood cell parameters along with serum ferritin. Seventeen Patients with Serum ferritin levels above 30 ng/ml were included in the study for further analysis of hematological parameters and clinical parameters after phase I therapy at the end of third month. Two patients dropped out during phase I therapy. Total No of 15 patients were considered finally for further treatment and investigation. The obtained results were tabulated and the collected data were subjected to statistical analysis.

STATISTICAL ANALYSIS:

The collected data were analysed statistically through (SPSS) Statistical Package for Social Science).

The Normality tests Kolmogorov-Smirnov and Shapiro-Wilks tests results revealed that except variable ESR (1/2 hour and 1 hour) values, all other variables follow Normal distribution, and ESR values do NOT follow Normal distribution. Therefore to analyse the data both Parametric and Non parametric methods were applied. For variables which follow Normal distribution, to compare mean values between Pre Op and Post Op **Paired samples t-test** was applied. To compare Pre Op and Post Op ESR values **Wilcoxon Signed Rank test** was applied. To analyse the data SPSS (IBM SPSS Statistics for Windows, Version 22.0, Armonk, NY: IBM Corp. Released 2013) was used. Significance level was fixed as 5% ($\alpha = 0.05$).

TABLE 1: MEAN CHANGE IN PLAQUE INDEX SCORES FROM BASELINE TO POST OPERATIVE:

Variable		N	Mean	Std. Dev	t-Value	P-Value
PLAQUE INDEX	Plaque Index: Pre Op	15	1.03760	.068172	4.085	.001
	Plaque Index: Post Op	15	.9673	.03751		

The mean plaque index score at baseline was (1.04 ± .06) and post-operative score was found to be (.97 ± .03). On comparing baseline score with post-operative score the reduction in the plaque index score was *found to be statistically significant* ***[P-Value<0 .001].***

CHART 1: MEAN CHANGE IN PLAQUE INDEX SCORES FROM BASELINE TO POST-OPERATIVE

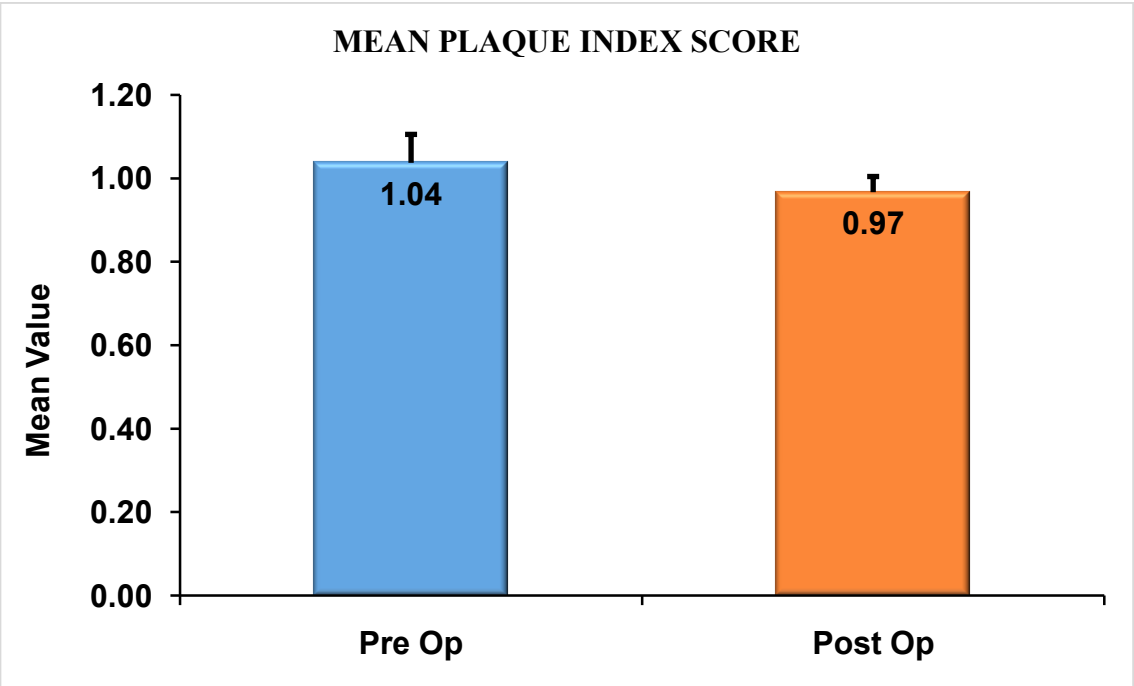


TABLE 2: THE MEAN CHANGE IN PERCENTAGE OF BLEEDING SITES FROM BASELINE TO POST OPERATIVE

Variable		N	Mean	Std. Dev	t-Value	P-Value
BLEEDING INDEX (%)	Bleeding Index: Pre Op	15	71.2513	19.05725	11.485	<0.001
	Bleeding Index: Post Op	15	42.7873	18.13282		

The mean bleeding sites at baseline was (71.25 ± 19.05 %) and post-operatively it was found to be (42.79 ± 18.13%) the reduction in bleeding sites percentage was found to be *Statistically Significant [**P-Value <0.001**]*

CHART 2: MEAN CHANGE IN THE PERCENTAGE OF BLEEDING SITES FROM BASELINE TO POST-OPERATIVE

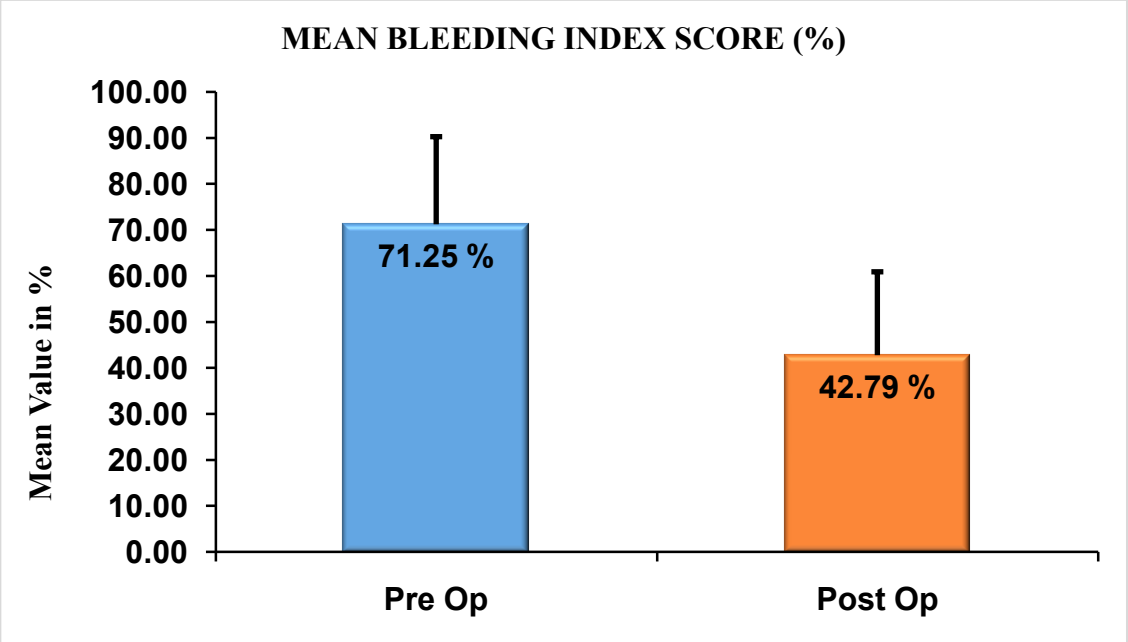


TABLE 3: THE MEAN CHANGE IN PROBING DEPTH AND CLINICAL ATTACHMENT LEVEL FROM BASELINE TO POST-OPERATIVE

Variables		N	Mean (mm)	Std. Dev	t-Value	P-Value
Probing Depth:	Baseline	15	4.79993	.553551	13.344	<0.001
	Post Op	15	3.74373	.614341		
Clinical Attachment Level:	Baseline	15	5.11673	.562817	15.849	<0.001
	Post Op	15	3.97827	.609834		

The mean probing depth and CAL at baseline was (4.79 ± .55 mm) and (5.12 ± .56 mm), after phase I therapy the probing depth and clinical attachment level was found to be (3.74 ± .61 mm) and (3.98 ± .60 mm) respectively. On comparing baseline probing depth and clinical attachment levels with post-operative probing depth and clinical attachment level the reduction in Probing depth and gain in Clinical attachment level post-operatively was found to be statistically significant with [***P-value <0.001***]

CHART 3: THE MEAN CHANGE IN PROBING DEPTH FROM BASELINE TO POST-OPERATIVE

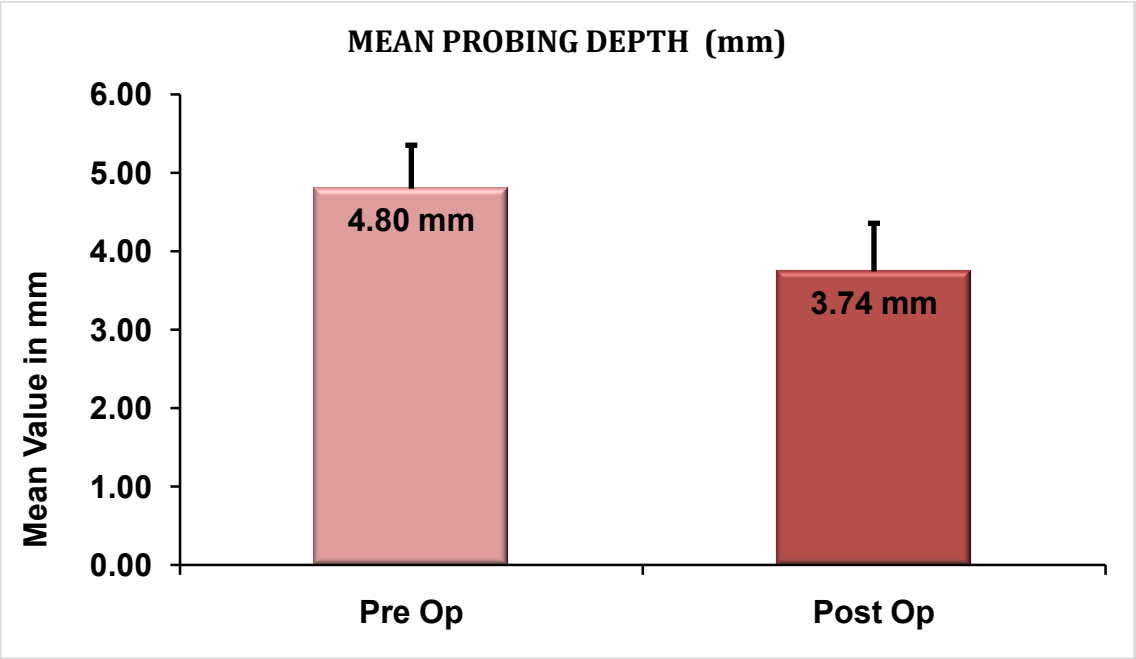


CHART 4: THE MEAN CHANGE IN CLINICAL ATTACHMENT LEVEL FROM BASELINE TO POST-OPERATIVE

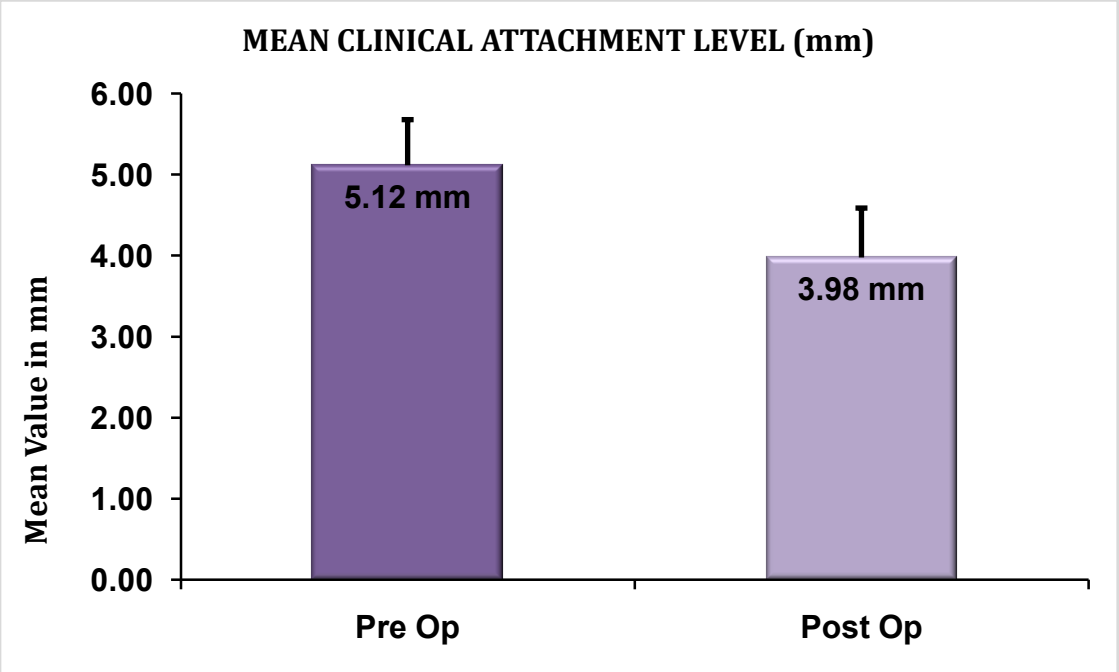


TABLE 4: MEAN CHANGE IN RBC COUNTS FROM BASELINE TO POST-OPERATIVE.

Variable		N	Mean	Std. Dev	t-Value	P-Value
Red Blood Cell count (million/cu mm)	Pre Op	15	4.4753	.54086	4.610	<0.001
	Post Op	15	4.9787	.61752		

The mean RBC value at baseline was (4.48 ± .54) millions/cu mm and post-operative value was (4.98 ± .61) millions/cu mm. On comparing both the values, the increase in RBC count post-operatively was found to be statistically significant with [***P-value <0.001]***

CHART 5: MEAN CHANGE IN RBC COUNT FROM BASELINE TO POST-OPERATIVE

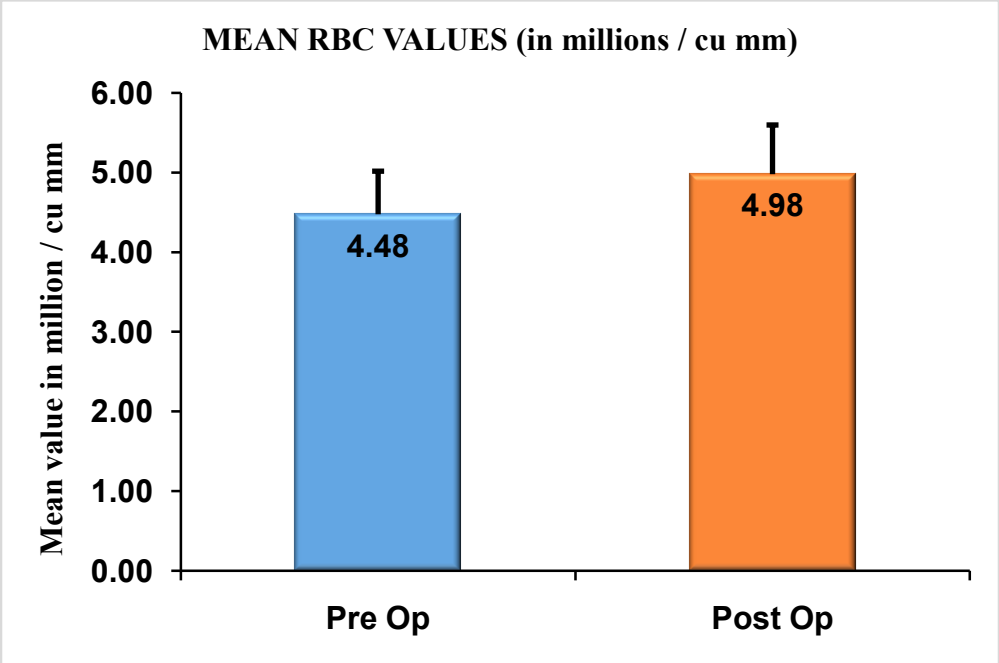


TABLE 5: THE MEAN CHANGE IN HEMOGLOBIN COUNTS FROM BASELINE TO POST-OPERATIVE

Variable		N	Mean	Std. Dev	t-Value	P-Value
Haemoglobin (Percentage)	Pre Op	15	12.7327	1.22915	4.740	<0.001
	Post Op	15	14.0480	1.30395		

The mean hemoglobin concentration at baseline (12.73 ± 1.22 %) and post-operatively was (14.04 ± 1.30 %). On comparing both values the increase in Hb concentration post-operatively was statistically significant with [***P-value <0.001***]

CHART 6: MEAN CHANGE IN HEMOGLOBIN CONCENTRATION FROM BASELINE TO POST-OPERATIVE

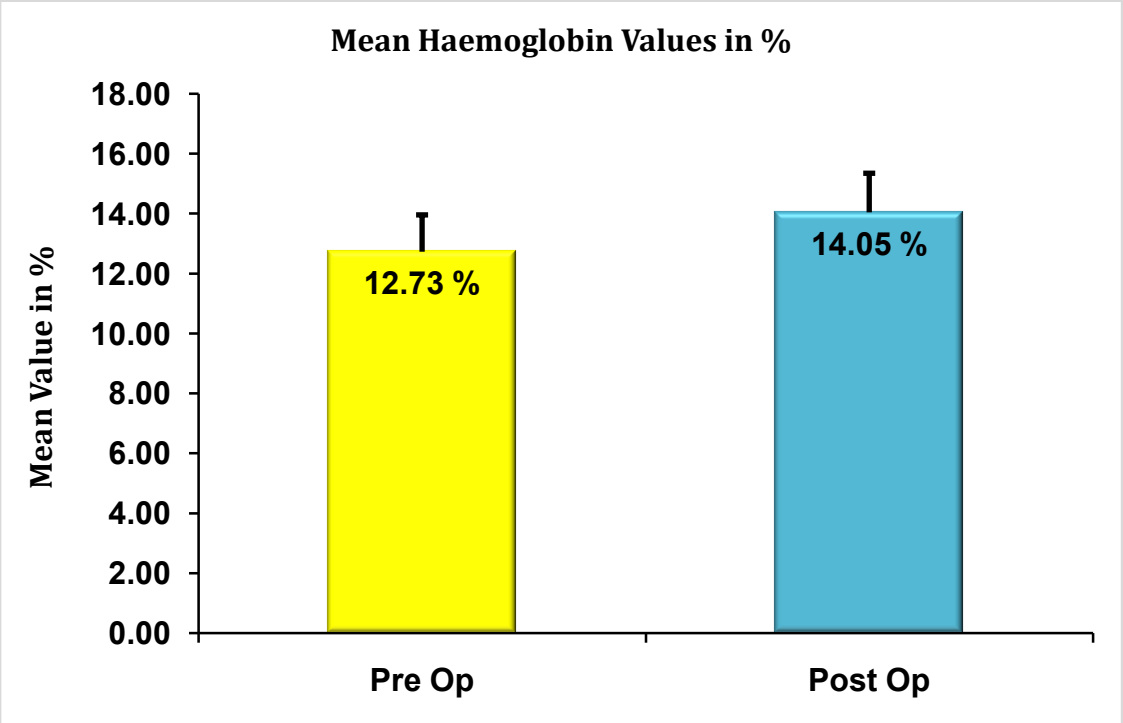


TABLE 6: MEAN CHANGE IN MCV VALUE FROM BASELINE TO POST- OPERATIVE

Variables		N	Mean	Std. Dev	t-Value	P-Value
MCV (fL)	Pre Op	15	87.70	10.69	.571	.572
	Post Op	15	89.69	8.23		

The mean change in MCV value from baseline to post-therapy was (87.70 ± 10.69 to 89.69 ± 8.23 fL) the increment in the post-operative value was not found to be statistically significant.

CHART 7: MEAN CHANGE IN MCV VALUE FROM BASELINE TO POST-TREATMENT:

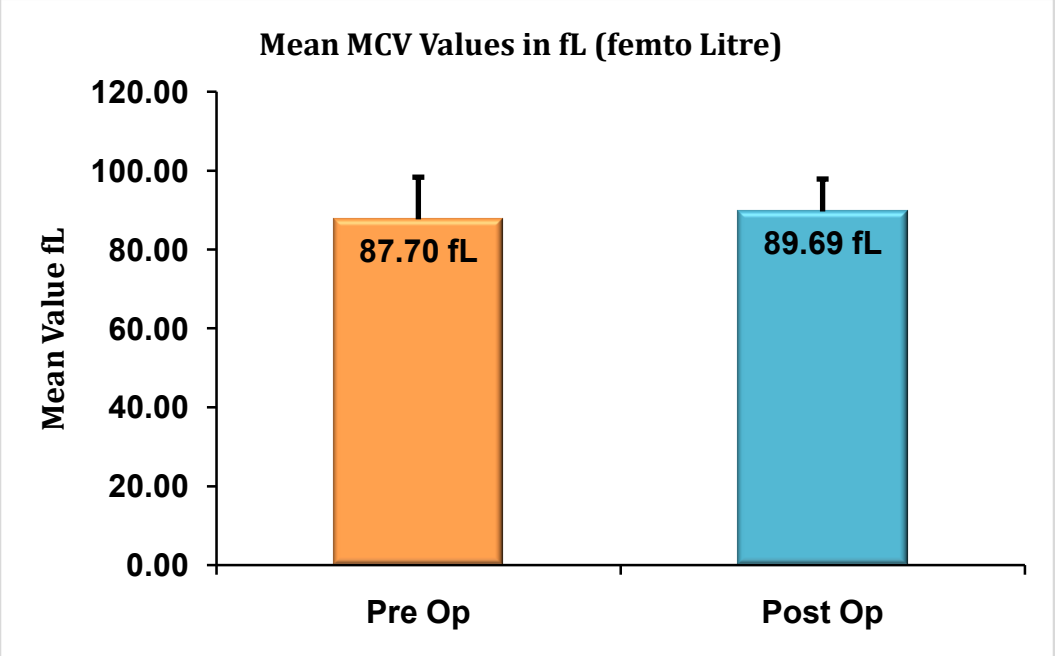


TABLE 7: MEAN CHANGE IN MCH VALUE FROM BASELINE TO POST- OPERATIVE

Variables		N	Mean	Std. Dev	t-Value	P-Value
MCH (pg)	Pre Op	15	28.27	2.07	-.536	.601
	Post Op	15	28.63	1.28		

The mean change in MCH value from baseline to post-treatment was (28.27 ± 2.07 to 28.63 ± 1.28 pg) the increase in postoperative value was not significant statistically.

CHART 8: MEAN CHANGE IN MCH VALUE FROM BASELINE TO POST-TREATMENT:

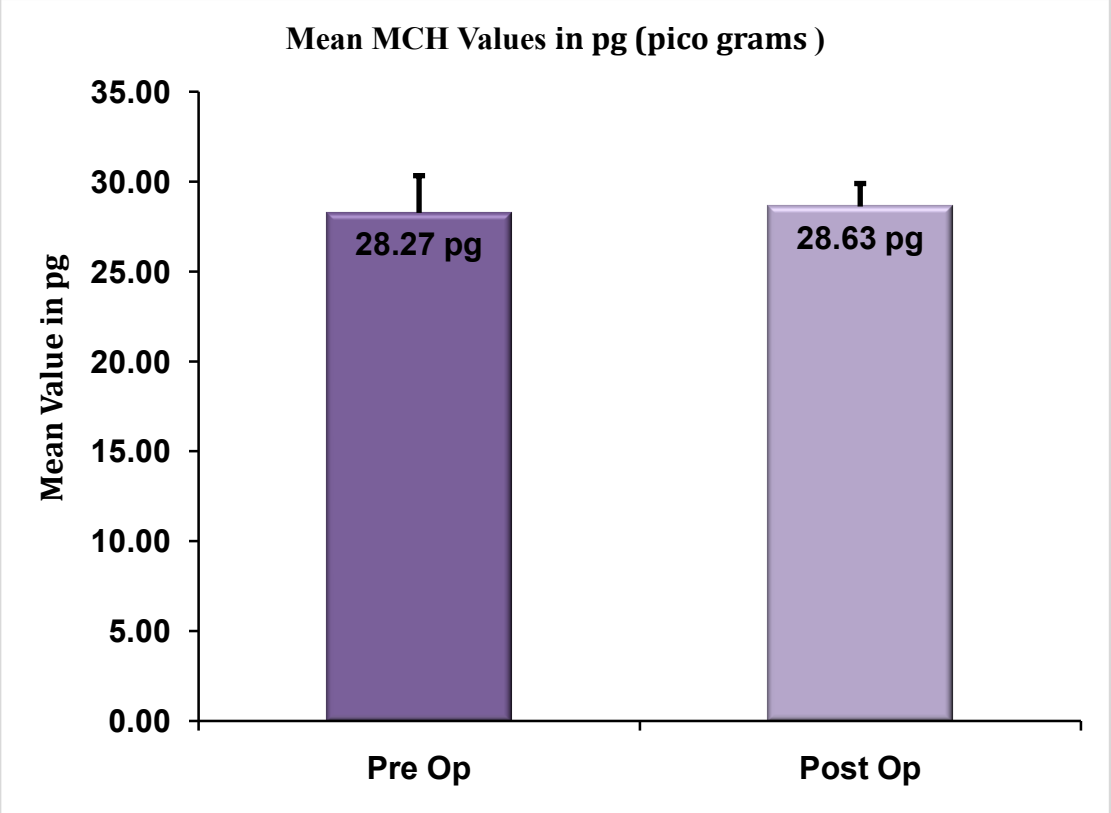


TABLE 8 : MEAN CHANGE IN MCHC VALUE FROM BASELINE TO POST-OPERATIVE

Variable		N	Mean	Std. Dev	t-Value	P-Value
MCHC (%)	Pre Op	15	29.99	5.32	.530	.600
	Post Op	15	30.89	3.86		

The mean change in MCHC value from baseline to post-therapy was (29.99 ± 5.32 to 30.89 ± 3.86 %) the increase was not found to be statistically significant.

CHART 9: MEAN CHANGE IN MCHC VALUE FROM BASELINE TO POST-TREATMENT

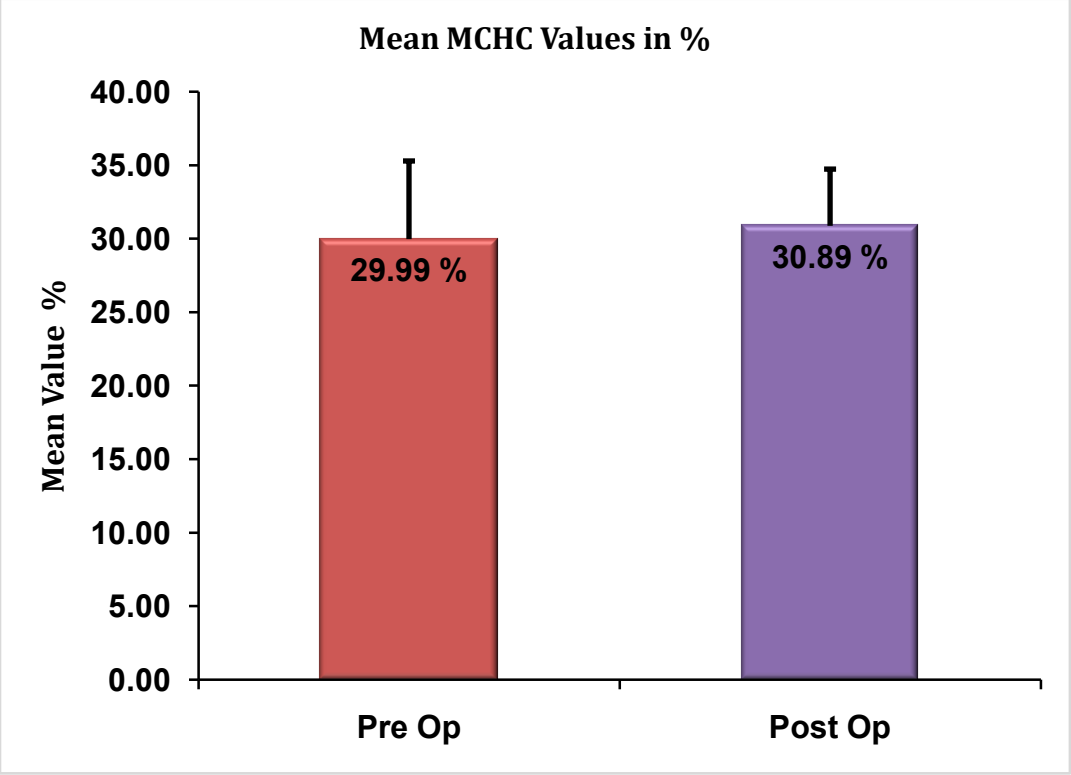


TABLE 9 : THE MEAN CHANGE IN PCV (PACKED CELL VOLUME) FROM BASELINE TO POST-OPERATIVE

Variables		N	Mean	Std. Dev	t-Value	P-Value
PCV / Hematocrit volume (%)	Pre Op	15	44.820	8.2487	-1.000	.334
	Post Op	15	47.693	8.5280		

The mean PCV percentage at baseline was (44.82 ± 8.25 %) and post-operatively (47.69 ± 8.53 %) respectively. On comparing these two mean values the increase in PCV percentage after therapy was not statistically significant with *P-value .334.*

CHART 10: THE MEAN CHANGE IN PCV PERCENTAGE FROM BASELINE TO 3 MONTH POST-OPERATIVE

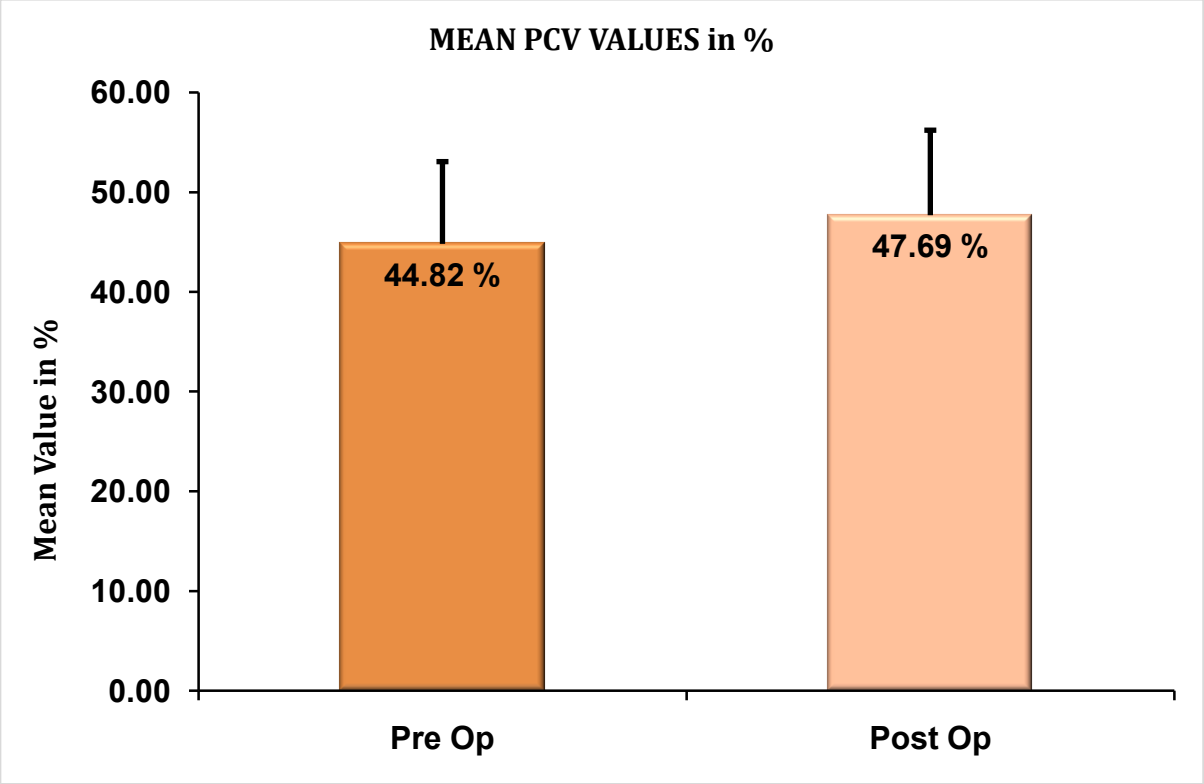


TABLE 10: THE MEAN CHANGE IN ESR VALUE FROM BASELINE TO POSTOPERATIVE AFTER HALF-AN HOUR AND ONE HOUR INTERVAL

Descriptive Statistics

		Pre Op	Post Op
ESR (1/2hr)	N	15	15
	Mean	8.47	4.07
	Std. Dev	6.010	2.549
	Median	7.0	3.0
	1st Quartile	3.0	2.0
	3rd Quartile	12.0	6.0
ESR(1hr)	N	15	15
	Mean	20.87	10.80
	Std. Dev	13.266	7.173
	Median	16.0	9.0
	1st Quartile	9.0	5.0
	3rd Quartile	36.0	18.0

Wilcoxon Signed Ranks Test to compare ESR values between Pre-Operative and Post-Operative values:

		P-Value
ESR (1/2hour): Post Op - ESR(1/2hour): Pre Op	Negative Ranks	0.001
	Positive Ranks	
ESR(1 hour): Post Op - ESR(1hour): Pre Op	Negative Ranks	0.001
	Positive Ranks	

The mean baseline ESR value at half-an hour was (8.47 ± 6.01) and post-operatively it was (4.07 ± 2.55), On comparing these two mean values the decrease in ESR value was found to be statistically significant with *P-value* <0.001.

The mean baseline ESR value at one hour was (20.87 ± 13.26) and post-operatively it was (10.80 ± 7.17), On comparing these values the decrease in ESR value post-therapy was found to be statistically significant with *P-value <0.001.*

CHART 11: THE MEAN CHANGE IN ESR VALUE AT HALF AN HOUR FROM BASELINE TO POST-TREATMENT

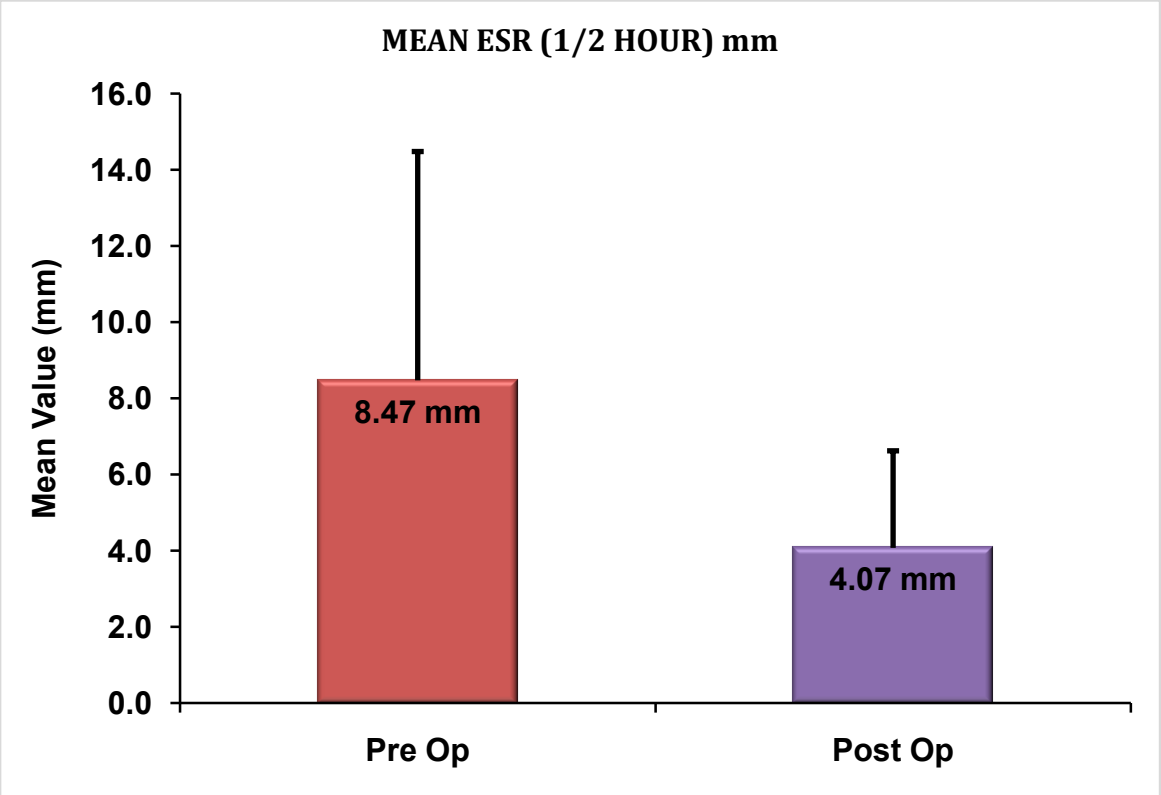
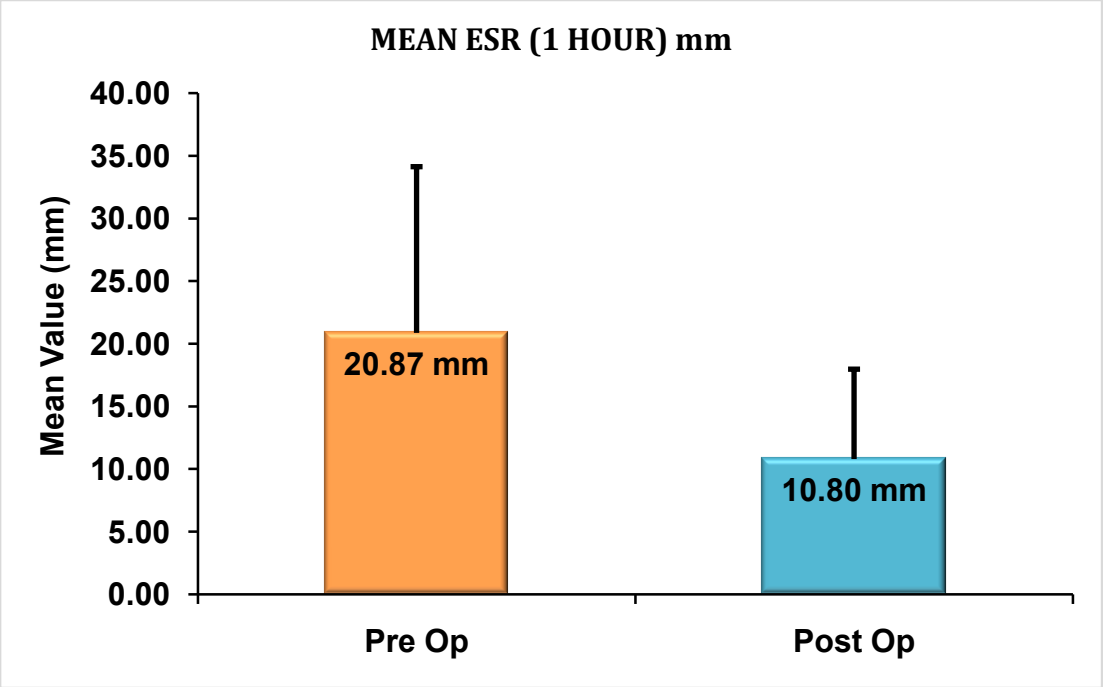


CHART 12: THE MEAN CHANGE IN ESR VALUE AT ONE HOUR FROM BASELINE TO POST-TREATMENT



DISCUSSION

An interventional study was conducted in Department of Periodontology, APDCH, Melmaruvathur, Tamilnadu. 30 Generalized Aggressive Periodontitis patients were enrolled in the study. A written informed consent was obtained from all the patients. Hematological parameters like (RBCs, Hb, PCV, ESR and Serum Ferritin) were evaluated at baseline along with the clinical indices (Plaque index, Bleeding index) and clinical parameters (Probing depth, Clinical attachment level). Among the thirty GAgP patients, thirteen patients had their serum ferritin levels less than 30 ng/ ml, which were found to be a positive indication of Iron deficiency. Seventeen Patients with serum ferritin above 30 ng /ml and Hb less than or equal to 15% were included in the study to evaluate the effect of periodontal therapy on Red blood cell parameter. All participants were allocated for Non-surgical periodontal therapy and further blood investigation and clinical parameters at the end of third month post operatively. Two patients failed to report, finally 15 patients were considered in the study.

The relationship between Anemia and Periodontal disease was explored in 20th century. Several studies reported the concept of Anemia as an etiologic factor for periodontitis and periodontitis as a risk factor for anaemia. Several studies confirmed the link between Anemia and Chronic Periodontitis **(Hutter *et al.,* Gokhale *et al.,* Naik *et al.,* Pradeep *et al.,* and Patel *et al.,*)**.Few studies also suggested

that periodontal therapy helps to reduce inflammation thereby improving anemic status in periodontitis patients **(Anumolu *et al.*, Shetty *et al.*, Agarwal *et al.*, Pradeep *et al.*,).**

The evidence for the relationship between AgP and Anemia is limited. **Anandh *et al.*,** in his case-control study suggested that like chronic periodontitis, GAgP also associated with a risk for Anemia. **Christan *et al.*,** conducted a prospective longitudinal study to examine the systemic effect of non-surgical therapy on WBC count and differential blood count in GAgP patients and concluded that periodontal therapy may significantly reduce leucocyte counts in patients with GAgP but reported mild difference between smokers and non-smokers.

This is the first intervention study conducted to evaluate the systemic markers related to anemia in GAgP patients before and after phase I periodontal therapy. Since aggressive periodontitis is presented with higher levels of circulating cytokines such as IL-1, IL-6, IL-17, TNF-α , INF-γ and other pro-inflammatory cytokines, which might also lead to a low grade systemic inflammation that would cause Anemia. In order to treat ACD, it's better to eliminate the cause either systemic or local thereby minimizing the treatment from invasive to conservative. Untreated anemia may cause a critical issue in patients with chronic disease[56]. In case of inflammatory disease like Periodontitis,

periodontal therapy is one such possible way to control the inflammation and reverse the condition.

Hence, the present study was conducted to investigate whether Aggressive periodontitis is associated with reduced Erythrocyte count and Hemoglobin levels, and like chronic periodontitis Aggressive periodontitis also respond to Phase I periodontal therapy.

The study results showed a significant reduction in the mean Plaque Index score from baseline to post-therapy (1.03 ± .06 to .96 ± .03). This may be due to the elimination of local factors which harbors numerous pathogens. There was a statistically significant reduction in the mean Bleeding sites percentage from baseline to post-therapy following Non-surgical phase (71.25 ± 19.05% to 42.79 ±1.13%). This may be due to the resolution of inflammation. Periodontal inflammation often causes bleeding from the gingiva, direct loss of blood from the gingiva might also be a reason for reduced RBC count but evidence has not been substantiated, few plausible mechanisms explained the decreased hematological parameters in periodontitis patients[30,34]. So the reduction in bleeding sites post-operatively would have improved the hematological variables. There was a significant reduction in the mean probing depth readings from baseline to 3 months after therapy (4.80 ± .55 mm to 3.74 ± .61 mm) . In case of increased probing pocket depth the subgingival organism and their products enter the blood stream and affect the distant sites through

ulceration of pocket epithelium that evoke low grade systemic inflammation, that have been related to suppression of erythropoiesis.[8] The reduction in probing depth post-therapy could be attributed to effective mechanical debridement as a result of which bacterial load and inflammation reduced. A significant gain in mean CAL value was seen from baseline to 3 months after therapy (5.12 ± .56 mm to 3.98 ± .60 mm) which might be due to subgingival scaling and root planing as it leads to resolution of inflammation and cessation of disease progression hence resulting in gain of attachment level. Following phase I therapy all participants received oral hygiene instruction for home care plaque reduction, not only the clinical parameters but also the hematological parameters were found to be improved from baseline to post-therapy.

The blood investigation results showed a statistically significant increase in the mean RBC count from baseline to post-operative (4.48 ± .54 to 4.98 ± .61) millions/cu mm. And the mean Hb change from pre-operative to post-operative (12.73 ± 1.22 % to 14.05± 1.30 %) which was also statistically significant. The low grade systemic inflammatory burden was suggested as one of reasons for defective red cell production in spite of adequate iron stores. Once the elimination of inflammatory components are done the suppression of erythropoeisis is prevented thereby improvement in anemic status is achieved. The increase in values of HB% and RBC count post treatment was supported by many studies done in chronic periodontitis and the

results were in accordance with the study done by **Malhotra** *et al*., in chronic periodontitis patients. Since there is very few studies done in GAgP related to anemia and no interventional study so far was done, hence the study results were not compared with any other studies.

The mean improvement in Haematocrit (PCV) percentage from baseline to 3 months post operatively (44.82 ± 8.25% to 47.69 ± 8.53%) was found to be statistically non-significant; this may be due to small sample size. The increase in the blood indices (MCV, MCH, and MCHC) value post treatment was not very high. The mean changes in the blood indices values were not statistically significant. The baseline blood indices values were between the normal or near normal range that indicated the mild anemia was not due to iron or vitamin deficiency.

In general, the reduction in the MCV and MCH value suggests microcytosis which is commonly seen in iron deficiency and elevated levels of MCV and MCH value suggests macrocytosis caused by vitamin deficiency. In this study the MCV and MCH values were in normal reference range with small increment seen post-operatively indicating the Anemia to be normocytic as seen in ACD. Even though the haemoglobin concentration at baseline was found to be reduced the MCHC was not below the normal reference ranges in both the groups at baseline and post-therapy, indicating that Hb concentration per volume of packed red cell is normal. The normal MCHC reference range indicates normochromic anemia as seen in ACD.

Another valuable parameter indicating the underlying inflammatory process was the ESR value. The elevated ESR value at baseline suggested that Aggressive Periodontitis has an inflammatory component systemically and the reduction in ESR value after periodontal therapy over time suggested resolution of inflammation and inflammatory markers. ESR estimation is the oldest laboratory methods that help to monitor disease activity and reflects the systemic response of any chronic disease and results from a change in colloidal state of plasma that could be caused by variation in plasma proteins globulins and fibrinogens.[1]

Aggressive periodontitis patients have elevated ESR when compared to chronic periodontitis and healthy individuals. The mean change in ESR value at one hour from baseline to post-therapy was statistically significant (20.87 ± 13.27 mm to 10.80 ± 7.173 mm). This statistically significant reduction of ESR value post-therapy might be due to the reduction in the systemic disease activity.

The results indicated that the RBC count and Hb concentration were significantly increased after therapy with a significant decrease in ESR value and minimal increment was observed in the PCV, MCH, MCV and MCHC. Clinical parameters showed a statistically significant improvement after therapy. Few limitations of this present study were the relatively smaller sample size and variables like socioeconomic status and stress were not elucidated.

CONCLUSION

As the saying goes “Mouth is the mirror of health and disease”. The concept that periodontal diseases are confined to tooth and supporting structures has been revised, as it can exaggerate to cause wide ranging systemic effects. Aggressive periodontitis being one of the clinical entity that can cause progressive destruction of periodontium and loss of alveolar bone. This might lead to increased levels of circulating pro-inflammatory cytokines. This low grade systemic inflammation might cause depletion of RBC production and anaemia to a milder extent when compared to other chronic inflammatory disease like rheumatoid arthritis.

The main aim of the present study was to evaluate the systemic markers related to anaemia in GAgP patients before and after phase I therapy. The results of this present study showed,

1) A statistically significant improvement in the clinical parameters were observed such as reduction in **Plaque index score, Percentage of Bleeding sites, Probing depth** and gain in **Clinical attachment level** after Phase I periodontal therapy in GAgP patients.
2) A statistically significant improvement in **Erythrocyte count and Haemoglobin concentration** was observed after Non-surgical periodontal therapy in GAgP patients.

3) Following Phase I therapy a very minimum increment in the **MCH, MCV, MCHC** value was observed which was not found to be statistically significant, the small increment in the value suggesting Normocytic Normochromic Anemia as seen in ACD.
4) The improvement in the PCV percentage after non-surgical therapy was not found to be statistically significant. This may be due to smaller sample size.
5) A statistically significant decrease in the **Erythrocyte Sedimentation Rate** was observed after therapy indicating a reduction in the systemic infection.

Within the limitations of this present study it can be concluded that like other inflammatory disease, Aggressive periodontitis also exaggerate a mild systemic inflammation that might cause ACD and like chronic periodontitis, Generalized Aggressive Periodontitis also respond to Non-surgical periodontal therapy and provides evidence in the improvement of anemic status.

REFERENCES

1. Anand PS, Sagar DK, Ashok S, Kamath KP. Association of aggressive periodontitis with reduced erythrocyte counts and reduced hemoglobin levels. *Journal of Periodontal Research*. 2014;49(6):719-28.
2. Lainson PA, Brady PP, Fraleigh CM. Anemia, a systemic cause of periodontal disease?. *J Periodontol*. 1968;39(1):35-8.
3. Weiss G, Goodnough LT. Anemia of chronic disease. *New England Journal of Medicine*. 2005;352(10):1011-23.
4. Agarwal N, Kumar VS, Gujjari SA. Effect of periodontal therapy on hemoglobin and erythrocyte levels in chronic generalized periodontitis patients: An interventional study. *Journal of Indian Society of Periodontology*. 2009;13(1):6.
5. Hutter JW, Velden UV, Varoufaki A, Huffels RA, Hoek FJ, Loos BG. Lower numbers of erythrocytes and lower levels of hemoglobin in periodontitis patients compared to control subjects. *Jclinperiodontol*. 2001;28(10):930-6.
6. Muppalla C, Theyagarajan R, Ari G, Mahendra J. evaluation of systemic markers related to anemia in peripheral blood of patients with chronic generalised severe periodontitis a comparative study. *International Journal of Current Research and Review*. 2016;8(9):59.

7. Li X, Kolltveit KM, Tronstad L, Olsen I. Systemic diseases caused by oral infection. *Clinical microbiology reviews*. 2000;13(4):547-58.
8. Malhotra R, Kapoor A, Grover V, Grover D, Kaur A. Effect of scaling and root planing on erythrocyte count, hemoglobin and hematocrit in patients with chronic periodontal disease. *American Dental Hygienists Association.* 2012;86(3):195-203.
9. Newman MG, Takei H, Klokkevold PR, Carranza FA. Classification of diseases and conditions affecting periodontium. Carranza's clinical periodontology. Elsevier health sciences; 2011 Feb 14.
10. Kotwal B, Mahajan N, Kalvani H, Dewan M. Non-surgical periodontal therapy–Revisited. *IOSR J Dent Med Sci*. 2013;9:15-9.
11. Walker BR, Colledge NR. Davidson's Principles and Practice of Medicine E-Book. Elsevier Health Sciences; 2013 Dec 6.
12. Mohan H. Textbook of pathology. New Delhi: Jaypee brothers medical publishers; 2005.
13. Santosh HN, Tejavathi Nagaraj AS. Anemia of chronic disease: A comprehensive review. *Journal of Medicine, Radiology, Pathology & Surgery*. 2015;1:13-6.
14. Jurado RL. Iron, infections, and anemia of inflammation. *Clinical Infectious Diseases*. 1997 Oct 1;25(4):888-95.
15. Means RJ, Krantz SB. Progress in understanding the pathogenesis of the anemia of chronic disease [see comments]. *Blood*. 1992;80(7):1639-47.

16. Weinstein DA, Roy CN, Fleming MD, Loda MF, Wolfsdorf JI, Andrews NC. Inappropriate expression of hepcidin is associated with iron refractory anemia: implications for the anemia of chronic disease. *Blood*. 2002;100(10):3776-81.
17. Atkins MB, Kappler K, Mier JW, Isaacs RE, Berkman EM. Interleukin-6-associated anemia: determination of the underlying mechanism. *Blood*. 1995 Aug 15;86(4):1288-91.
18. Zarychanski R, Houston DS. Anemia of chronic disease: A harmful disorder or an adaptive, beneficial response?. *Canadian Medical Association Journal*. 2008 Aug 12;179(4):333-7.
19. Duarte PM, Rocha M, Sampaio E, Mestnik MJ, Feres M, Figueiredo LC, Bastos MF, Faveri M. Serum levels of cytokines in subjects with Generalized chronic and Aggressive periodontitis before and after Non-surgical Periodontal Therapy: A Pilot study. *J Periodontol* 2010;81(7):1056-63
20. Patel MD, Shakir QJ, Shetty A. Interrelationship between chronic periodontitis and anemia: A 6-month follow-up study. *Journal of* Indian Society of Periodontology. 2014: 18(1):19-24.
21. Cash MJ, Sears DA. The Anemia of chronic disease: Spectrum of Associated Diseases in a series of unselected Hospitalized Patients. *The American Journal of Medicine.* 1989;87:638-43.
22. Greenstein G. Periodontal Response to Mechanical non-surgical therapy: A Review*. *J Periodontol* 1992;63(2):118-27.
23. Loos BJ, Craandjik J, Hoek FJ, Wertheim-van Dillen, Uele van der Velden. Elevation of systemic markers related to cardiovascular

Diseases in the peripheral Blood of Periodontitis Patients. *J Periodontol* 2000;71(10):1528-34.

24. Christen C, Dietrich T, Hagewald S, Kage A, Bernimoulin J-P: White blood cell count in generalized aggressive periodontitis after non-surgical therapy. *J clin Periodontol* 2002;29:201-6
25. Loos BG. Systemic markers of inflammation in Periodontitis. *J Periodontol* 2005;76(11):2106-13.
26. Havemose-Poulsen A, Westergaard J, Stoltze K, Skjodt H, Danneskiold-Samsoe B, Locht H, Bendtzen K, Holmstrup P. Peiodontal and Haematological characteristics associated with Aggressive Periodontitis, Juvenile Idiopathic Arthritis, and Rheumatoid Arthritis. *J Periodontol* 2006;77(2):280-7.
27. Erdemir EO, Nalcaci R, Caglayan O. Evaluation of systemic markers related to anemia of chronic disease in the peripheral blood of smokers and non-smokers with chronic periodontitis. *European Journal of Dentistry*. 2008;2:102-7.
28. Shi D, Meng H, Xu L, Zhang L, Chen Z, Feng X, Lu R, Sun X, Ren X. Systemic inflammation markers in patients with Aggressive Periodontitis: A Pilot study. *J Periodontol* 2008;79(12):2340-6.
29. Alijohani HA. Association between Hemoglobin level and severity of chronic periodontitis. *JKAU. Med. Sci*., 2009;17(1):53-60.
30. Gokhale SR, Sumanth S, Padhye AM. Evaluation of blood parameters in patients with chronic periodontitis for signs of Anemia. *J Periodontol* 2010;81(8):1202-6.

31. S.Y.Lu, H.L.Eng. Dramatic recovery from severe anemia by resolution of severe periodontitis. *J Dent Sci* 2015;5(1):41-6.

32. Naik V, Acharya A, Vijay L. Deshmukh, Shetty S, Shirhatti R. Generalized severe, chronic periodontitis is associated with anemia of chronic disease: a pilot study in urban Indian males. *Jornal of Investigative and clinical Dentistry* 2010;1:139-43.

33. Pradeep A.R, Anuj S, Arjun Raju P. Anemia of chronic disease and chronic periodontitis: Does Periodontal therapy Have an effect on Anemis status?. *J Periodontol* 2011;82(3):388-94.

34. Yamamoto T, Tsuneishi M, Furuta M, Ekuni D, Morita M, Hirata Y. Relationship between decrease of erythrocyte count and progression of periodontal disease in a Rural Japanese Population. *J Periodontol* 2011;82(1):106-13.

35. Garg B, Vardhan H, DE AK, Sahay AP. Estimation of serum ferritin- A Better Screening test for Blood Donors. *J Medicine* 2012;13:174-8.

36. Virdi HK. Hematological Parameters- A Diagnostic Mirror for Periodontitis. *Indian Journal of Dental Sciences.*2013;5(2):45-8.

37. Liu J, Zhao J, Li C, Yu N, Zhang D, Pan Y. Clinical and microbiological effect of nonsurgical periodontal therapy on patients with chronic or aggressive periodontitis. *Quintessence International Periodontology*. 2013;44(8):575-83.

38. Kalburgi NB, Koregol AC, Muley A, Warad S, Patil S. Anemia of chronic disease and tobacco use: An association based on

Hematological parameters. *International Journal of oral and maxillofacial pathology* 2013;4(2):18-23.

39. Nair SK, Faizzuddin M, Jayanthi D. Anemia and Periodontitis: An enigma? *IOSR Journal of Dental and Medical Sciences* 2013;11(4):71-8.
40. Jenebian N, Sattari FD, Salar N, Bijani A, Ghasemi N. The relation between Periodontitis and Anemia associated parameters. *Journal of Dentomaxillofacial Radiology, Pathology and Surgery*. 2013;2(3):26-32.
41. Kolte RA, Kolte AP, Deshpande NM. Assessment and comparison of anemia of chronic disease in healthy subjects and chronic Periodontitis patients: A clinical and hematological study. *Journal of* Indian society of Periodontology 2014;18((2):183-6.
42. Shetty MK, Thomas B, Shetty AV. Comparative evaluation of hemoglobin level in anemic patients with chronic periodontitis before and after treatment. *Journal of Interdisciplinary Dentistry* 2014;4(1):24-6.
43. Kundu Debabrata, BandyopadhyayP,Nair V, Chowdhury M, Mukherjee S, Nayek M. Aggressive periodontitis: A clinico-hematological appraisal. *Journal of* Indian society of Periodontology 2014:18(2):166-71.
44. Chakraborty S, TewariS,Sharma RK, Narula SC. Effect of Non-Surgical Periodontal therapy on serum ferritin levels: An Interventional study. *J Periodontol* 2014;85(5):688-95.

45. Mishra P, Agarwal S, Devraj C.G, Nayak P A, Yadav A, Sharma S. Determination of erythrocyte parameters in chronic periodontitis patients. *International Journal of Medical Science and Reasearch* 2014;1(3):148-53.

46. Kaur N, Goyal G, Padda S, Kaur B, Sunidhi. Iron deficiency anemia and oral health prospective- A Review. *Indian Journal of Comprehensive Dental Care* 2015;5(2):655-60.

47. Joshipura V, Yadalam U, Brahmavar B. Aggressive Periodontitis: A review. *Journal of International Clinical Dental Research Organization* 2015;7(1):11-6.

48. Santosh HN, Chaya M David, Kumar H, Sanjay CJ, Bose A. Chronic periodontitis and anemia of chronic disease: an observational study. *Arch Orofac Sci* 2015;10(2):57-64.

49. Khan NS, Luke R, Soman RR, Krishna PM, Safar IP, Swaminathan SK. Qualitative assessment of red blood cell parameters for signs of anemia in patients with chronic periodontitis. Journal of *International Society of Preventive and Community Dentistry*. 2015;5(6):476-81.

50. Anumolu V, Srikanth A, Paidi K. Evaluation of the relationship between anemia and periodontitis by estimation of blood parameters: A cross-sectional study. *Journal of* Indian Society of Periodontology 2016;20(3):265-72.

51. Löe H. The gingival index, the plaque index and the retention index systems. *J Periodontol*. 1967;38(6):610-6.

52. Wei SH, Lang KP. Periodontal epidemiological indices for children and adolescents: I. Gingival and periodontal health assessments. *Pediatr Dent*. 1981;3(4):353-60.

53. Barrett KE, Barman SM, Boitano S, Brooks H. Ganong's Review of Medical Physiology, 25/e.

54. Hall JE. Guyton and Hall Textbook of Medical Physiology E-Book. Elsevier Health Sciences; 2015 May 31.

55. Banerji A, Dey D, Baerjee P, Ray S, Ray R, Hazra B. CLSI-Derived Hematology Reference Intervels for healthy males in eastern india. *Global Journal of Medicine and Public Health* 2013;2(2):1-7.

56. Cavill I, Auerbach M, Bailie GR, Barrett-Lee P, Beguin Y, Kaltwasser P, Littlewood T, Macdougall IC, Wilson K. Iron and the anemia of chronic disease: a review and strategic recommendations. *Current medical research and opinion*. 2006;22(4):731-7.

PROFORMA

EVALUATION OF SYSTEMIC MARKERS RELATED TO ANEMIA IN THE PERIPHERAL BLOOD OF GENERALIZED AGGRESSIVE PERIODONTITIS PATIENTS BEFORE AND AFTER PHASE I PERIODONTAL THERAPY – AN INTERVENTIONAL STUDY

Name: Age/ Gender:

OP.No: Date:

Occupation: Address:

Chief complaint:

History of presenting illness:

Past medical history:

Past dental history:

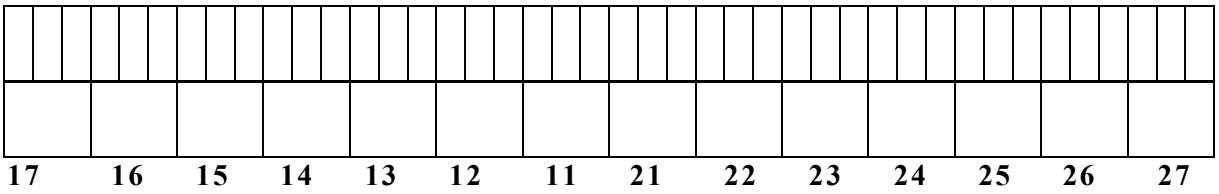
Family history:

Intra-Oral examination:

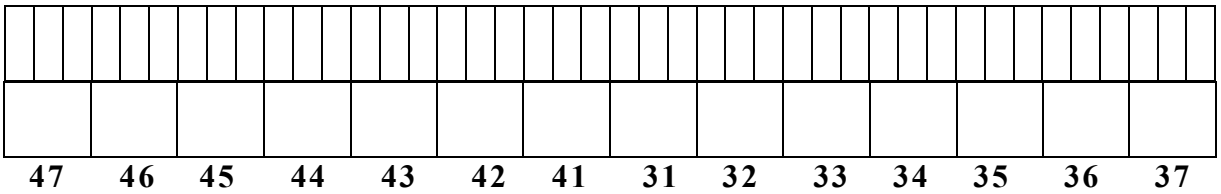
PERIODONTAL PARAMETERS:

PLAQUE INDEX: (Silness J. and Loe H. 1964)

BASELINE:



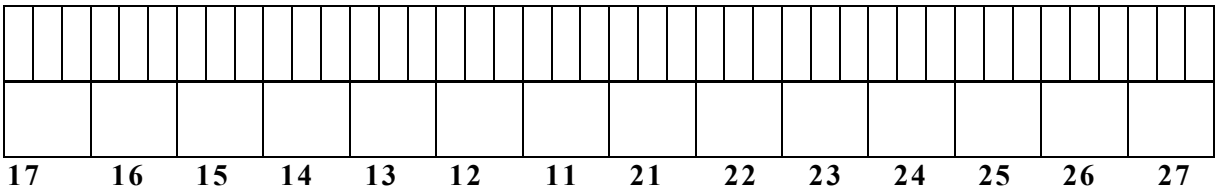
17 16 15 14 13 12 11 21 22 23 24 25 26 27



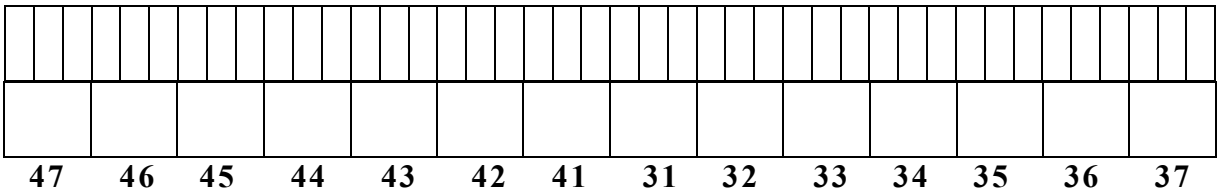
47 46 45 44 43 42 41 31 32 33 34 35 36 37

SCORE = ☐ **Excellent/Good/Fair/Poor**

3rd MONTH:



17 16 15 14 13 12 11 21 22 23 24 25 26 27



47 46 45 44 43 42 41 31 32 33 34 35 36 37

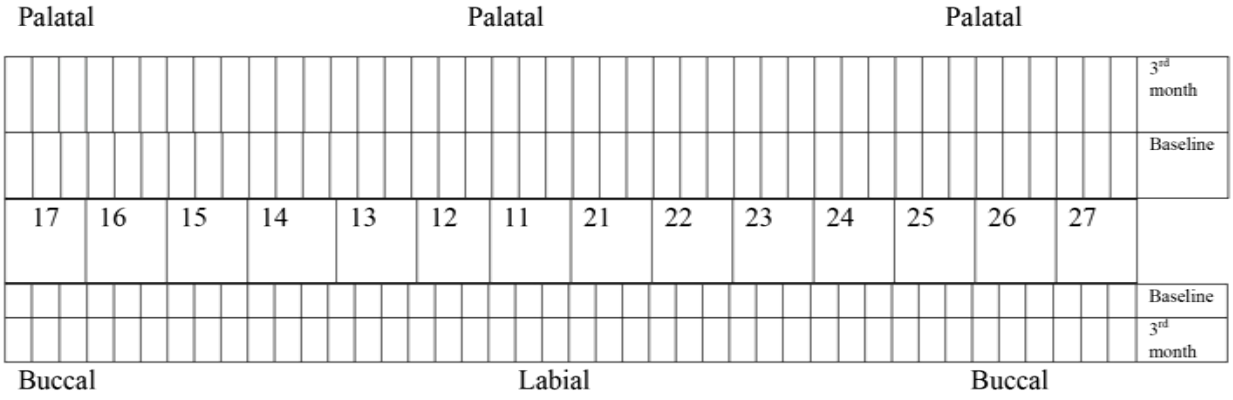
SCORE = ☐ **Excellent/Good/Fair/Poor**

GINGIVAL BLEEDING INDEX

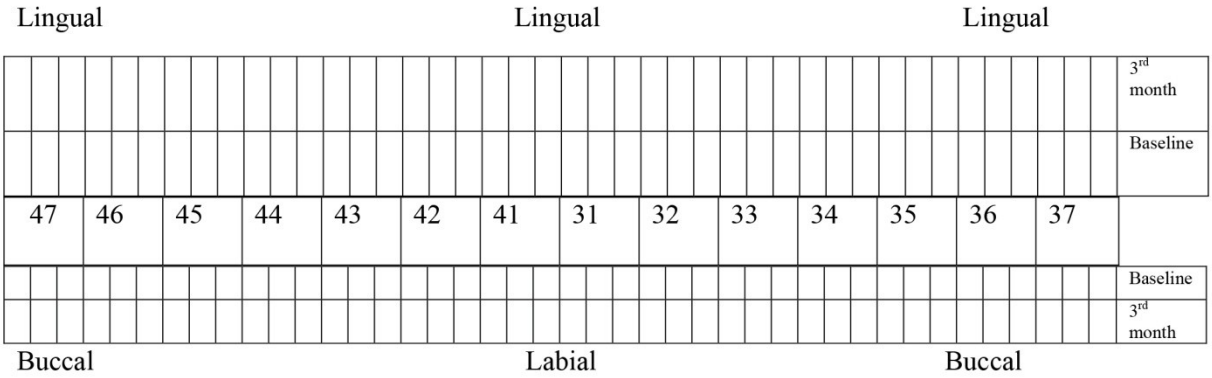
3rd MONTH														
BASELINE														
	7	6	5	4	3	2	1	1	2	3	4	5	6	7
BASELINE														
3rd MONTH														

PROBING DEPTH:

UPPER ARCH



LOWER ARCH



CAL:

3rd MONTH														
BASELINE														
	7	6	5	4	3	2	1	1	2	3	4	5	6	7
BASELINE														
3rd MONTH														

DIAGNOSIS:

HEMATOLOGICAL PARAMETERS

INVESTIGATIONS	**RESULTS**	
	PRE-OP (BASELINE)	**POST-OP (3rd MONTH)**
RBC COUNT		
HB%		
MCV		
MCH		
MCHV		
PCV		
ESR		

SERUM FERRITIN	**RESULT**

TREATMENT

Date	Treatment done	Appointment date	Staff signature

PARTICIPANT INFORMED CONSENT FORM (PICF)

(English)

Protocol / Study number: _____

Participant identification number for this trial: _____

The contents of the information sheet dated that was provided have been read carefully by me / explained in detail to me, in a language that I comprehend, and I have fully understood the contents. I confirm that I have had the opportunity to ask questions.

The nature and purpose of the study and its potential risks / benefits and expected duration of the study, and other relevant details of the study have been explained to me in detail. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal right being affected.

I understand that the information collected about me from my participation in this research and sections of any of my medical notes may be looked at by responsible individuals from APDCH. I give permission for these individuals to have access to my records.

I agree to take part in the above study.

---------- ----------

(Signatures / Left Thumb Impression) Signatures of the Principal Investigator

<u>ஆராய்ச்சியில் பங்கேற்பதற்கு இணக்கம்</u>

தேதி:

நோயாளியின் பெயர் :

வயது / பாலினம் :

புறநோயாளி எண் :

அறுவை சிகிச்சை மருத்துவ நிபுணரின் பெயர் :

சிகிச்சையின் பெயர் :_____

அளிக்கப்படும் மயக்க மருந்தின் வகை:

எனது தற்போதைய வாய் நலம் குறித்தும், அதற்கு உரிய சிகிச்சை முறைகளையும், மாற்று சிகிச்சை முறைகளையும் மற்றும் சிகிச்சை மேற்கொள்ளாவிடில் ஏற்படும் பின்விளைவுகளையும் பல்மருத்துவர் முழுமையாக என்னிடம் கூறினார். அதற்கான எனது சந்தேகங்களையும் பல்மருத்துவரிடம் கேட்டு தெளிவுபடுத்தி கொண்டேன். மேலும் சிகிச்சை முறை, என் சிகிச்சையின் போது தேவைப்படும் மயக்க மருந்துகள் மற்றும் பிற மருந்துகள் செலுத்த சம்மதிக்கின்றேன். நான் மனப்பூர்வமாக எனது சிகிச்சை முறை மற்றும் அதனால் வரும் பின்விளைவுகளையும் ஏற்றுக்கொள்கிறேன் மற்றும் மருத்துவர் கூறும் அறிவுரைகளையும் கடைபிடிப்பேன்.

மேலே சொல்லப்பட்டு இருக்கும் ஆராய்ச்சி ஆய்வில் பங்கேற்பதற்கு மனப்பூர்வமான எனது சம்மதம்.

மேலுள்ள தகவல்கள் உள்ளிட்டு ஆராய்ச்சி ஆய்வானது வாய் வழியாக விளக்கப்பட்டிருக்கிறது மற்றும் பங்கேற்பதற்கு சுய விருப்பத்தில் இ ணங்குகிறேன் என்பது இந்த ஆவணத்தில் கையெழுத்திடுவதன் அர்த்தமாகும்.

நோயாளியின் கையொப்பம் அறுவை சிகிச்சை நிபுணரின் கையொப்பம்

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ADHIPARASAKTHI DENTAL COLLEGE AND HOSPITAL

Melmaruvathur, Tamilnadu-603019

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This ethical committee has undergone the research protocol submitted by **SHOBANA . P** Post Graduate Student, Dept of PERIODONTICS under the title "**EVALUATION OF SYSTEMIC MARKERS RELATED TO ANEMIA IN THE PERIPHERAL BLOOD OF GENERALIZED AGGRESSIVE PERIODONTITIS PATIENTS BEFORE AND AFTER PHASE I PERIODONTAL THERAPY – AN INTERVENTIONAL STUDY",** Reference No**: 2015-MD-Br II-VID-05/APDCH** under the guidance of **DR. T. RAMAKRISHNAN** for consideration of approval to proceed with the study.

This committee has discussed about the material being involved with the study, the qualification of the investigator, the present norms and recommendation from the Clinical Research scientific body and comes to a conclusion that this research protocol fulfils the specific requirements and the committee authorizes the proposal.

Date:

CHAIR PERSON

- Inform IEC/IRB immediately in case of any issue(s) / adverse events.
- Inform IEC/IRB in case of any change of study procedure, site and investigator.
- Annual report to be submitted to IEC/IRB.
- Members of IEC/IRB have right to monitor the trial with prior intimation.