ESTIMATION OF SERUM ALBUMIN LEVELS IN CHRONIC GENERALISED PERIODONTITS, LOCALIZED AGGRESSIVE PERIODONTITIS PATIENTS AND COMPARE IT WITH PERIODONTALLY HEALTHY INDIVIDUALS

A Dissertation submitted

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for the degree of

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DEPARTMENT OF PERIODONTOLOGY
CERTIFICATE

This is to certify that **Dr.P.INDUMATHI,** Post graduate student (2016-2019) in the Department of Periodontics, Adhiparasakthi Dental College and Hospital, Melmaruvathur – 603319, has done this dissertation titled **"ESTIMATION OF SERUM ALBUMIN LEVELS IN CHRONIC GENERALISED PERIODONTITS,LOCALIZED AGGRESSIVE PERIODONTITIS PATIENTS AND COMPARE IT WITH PERIODONTALLY HEALTHY INDIVIDUALS"** under our direct guidance and supervision in partial fulfilment 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

TITLE OF THE DISSERTATION	Estimation of serum albumin levels in chronic generalised periodontits, localized aggressive Periodontitis patients and compare it with Periodontally Healthy individuals
PLACE OF THE STUDY	Adhiparasakthi Dental College and Hospital, Melmaruvathur-603319.
DURATION OF THE COURSE	3 Years
NAME OF THE GUIDE	Dr.T.Ramakrishnan, MDS.,
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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.

Co-guide **Guide & Head of Department**

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ABSTRACT

BACKGROUND:

Serum albumin is a negative acute phase protein, primarily synthesized by liver. Many factor affects the regulation of serum albumin levels. The primary ones involved are inflammation and malnutrition. Chronic disease which are associated with inflammation and the release of inflammatory cytokines such as interleukin-1, interleukin-6 and tumor necrosis factor α cause decrease in albumin. Periodontitis is a chronic inflammatory disease caused by bacterial infection of the supporting tissues around the teeth. A few studies have shown that serum albumin levels are reduced in patients with Generalised chronic Periodontitis.

AIM:

The aim of this study is to estimate and compare the serum albumin levels in chronic generalised and localized aggressive Periodontitis patients with that of Periodontally healthy individuals. To identify whether chronic generalised periodontitis and localized aggressive periodontitis have any effect on serum albumin levels compared to Periodontally healthy individuals.

MATERIALS AND METHODS:

Total number of 60 subjects were enrolled in this study with the age range of 18 -50 years. All subjects participating in the study were informed about the nature of the study and all individuals signed in the written informed consent form.

60 subjects were divided into 3 groups.

Group I- Periodontally healthy individual

Group II- Chronic generalised periodontitis

Group III- Localized aggressive periodontitis

Clinical parameters such as Gingival bleeding index(GI), Periodontal pocket depth (PPD), Clinical attachment level (CAL) and serum albumin level were evaluated between these three groups,

RESULTS:

All the clinical parameters shown to be increased significantly in patients with chronic generalised periodontitis (group II) and localized aggressive periodontitis (group III) when compared to periodontally healthy individuals (group I). There is a significant decrease in serum albumin level in patients with chronic generalised periodontitis (group II) and localized aggressive periodontitis (group III) when compared to periodontally healthy individuals (group I).

CONCLUSION:

With the limitations of the present study it could be concluded that there is inverse relationship of serum albumin level and periodontal disease. So, serum albumin level can be used as a predictive marker for severity of periodontitis. Further randomized clinical trials and longitudinal evaluations in a larger population would be required to support the observation of the present study.

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LIST OF ABBREVATIONS

APP	-	Acute-phase proteins
ALT	-	Alanine Aminotransferase
ALP	-	Alkaline Phosphatase
BCG	-	Bromocresol Green Albumin
BSA	-	Bovine Serum Albumin
BOP	-	Bleeding on probing
CAL	-	Clinical Attachment level
CRP	-	C-Reactive Protein
CDM	-	Chronic Kidney Disease
CEJ	-	Cemento-enamel junction
CVD	-	Cardiovascular Disease
CBS	-	Cystathionine β-synthase
GBI	-	Gingival Bleeding Index
GCP	-	Generalised Chronic Periodontitis
HSA	-	Human Serum Albumin
IL	-	Interleukin
IEC	-	Ion Exchange Chromatography
IAC	-	Immunoaffinity Chromatography
IMAC	-	Immobilized Metal-ion Affinity Chromatography
IFN	-	Interferon
LAP	-	Localized Aggressive Periodontitis
TNF	-	Tumor Necrosis Factor
TCA	-	Trichloroacetic acid
OVA	-	Ovalbumin
PD	-	Probing depth
UNC -15	-	University of North Carolina – 15

INTRODUCTION

Periodontitis is an infectious disease of gingival tissue origin, changes that occur in the bone are crucial as the alveolar bone destruction is responsible for tooth loss. Chronic periodontitis has been defined as *"*an infectious disease resulting in inflammation within the supporting tissues of the teeth, progressive attachment loss and bone loss*."*[1]

Aggressive periodontitis may be universally distinguished from chronic periodontitis by the age of the onset, the rapid rate of disease progression, the nature and composition of the associated subgingival microflora, alteration in the host immune response, and a familial aggregation of diseased individuals. Localized aggressive periodontitis affects primarily first molar and incisor teeth in adolescents with deep pockets.[1]

Serum albumin is a negative acute phase protein and serum albumin levels might be the practical marker of general health status (Phillips et al.)[2] Many conditions such as inflammatory states, liver diseases, and renal diseases have been indicated to reduce serum albumin levels (Herrmann et al.)[3]Inflammation and malnutrition both reduce serum albumin concentration by decreasing its rate of synthesis.[4] This suggests that periodontal disease severity might be indicated and monitored by the levels of serum albumin. Therefore, serum albumin can be used as a risk predictor for periodontal disease.

Studies demonstrated that the decreased serum albumin level is associated with patients with liver and renal disease.

Several studies have demonstrated that serum albumin concentrations are associated with general health status among elderly. Therefore, it becomes difficult to infer whether serum albumin concentrations are affected by an inflammatory component of periodontitis or the compromised nutritional status, owing to the general health status of the individual. This possibility of general health/nutritional status in any way affecting the serum albumin concentrations was somewhat eliminated in this study, as the subjects included in this study were physically fit without any systemic diseases. Thus, it would be legitimate to infer that the serum albumin concentration were solely affected by an inflammatory component of periodontitis.

Various studies have evaluated the levels of serum albumin with respect to chronic periodontitis and correlation with clinical parameters. But there is lagging evidence regarding serum albumin levels association with localized aggressive periodontitis. The purpose of this study is to estimate and compare the serum albumin levels in patients with clinically healthy gingiva, generalised chronic periodontitis and localized aggressive periodontitis.

AIM AND OBJECTIVES

The aim of this study is to estimate and compare the serum albumin levels in Chronic generalised and localized aggressive periodontitis patients with that of Periodontally healthy individuals

For this purpose, the following objectives were undertaken:

1. To identify whether chronic generalised periodontitis and localized aggressive periodontitis have any effect on serum albumin levels compared to periodontally healthy individuals.

2. To compare and correlate the clinical parameters such as gingival bleeding index, probing depth, CAL with serum albumin levels in patients with generalised chronic peiodontitis, localized aggressive periodontitis and periodontally healthy individuals.

GENERAL REVIEW

Acute-phase proteins (APP) defined as proteins whose serum concentration is altered at least 25% in response to inflammation and includes proteins of the complement, coagulation and fibrinolytic system, anti-proteases, transport proteins, inflammatory mediators and others.[5]

CLASSIFICATION[5]

They are classified as–

1. Positive acute phase proteins
2. Negative acute phase proteins

Positive APP increases with inflammatory response and negative APP are decrease in serum concentration with increase in inflammation.

Positive acute phase proteins

Positive acute phase proteins are C-reactive protein, Serum amyloid A, Serum amyloid P component, Complement factors, Mannan-binding lectin, Fibrinogen, prothrombin, factor VIII, Von Willebrand factor, Plasminogen, Alpha 2-macroglobulin, Ferritin, Ceruloplasmin, Haptoglobin, Alpha 1-antitrypsin and α1- antichymotrypsin and their functions are : Opsonisation of microbes, Recruitment of immune cells to inflammatory site, Induces enzymes which degrade the extra cellular matrix. Chemotaxis, lysis and clumping of target cells, Complement activation, Degradation of blood clots, Inhibits coagulation and

fibrinolysis by inhibiting thrombin. Binds hemoglobin and inhibits microbe iron uptake. Serpin down regulates inflammation[5].

Negative acute phase proteins

Negative acute phase proteins are Antithrombin, Albumin, Transferrin, Transthyretin, Transcortin, and Retinol – binding protein and their function includes increased coagulation, Increase free cortisol in blood, restoring homeostasis after stress.[5]

Regulation of the acute phase proteins production

Acute phase response is triggered by cytokines. Three main groups of cytokines that regulate the production of APP (Van Miert).

1. Cytokines act as positive or negative growth factors which includes, IL-2, IL-3, IL-4, IL-7, IL-10, IL-11, IL-12, granulocyte-macrophage colony stimulating factor.
2. Cytokines having pro-inflammatory properties are TNF-α/β, IL-1α/β, IL-6, IFN-α/γ, IL-8, macrophage inhibitory protein-1.
3. Factors accompanied by anti-inflammatory activity – IL-1 receptor antagonists, soluble IL-1 receptors, IL-1 binding protein, TNF-α binding protein.[5]

IL-1 considered as an important mediator of inflammation because of its presence at the inflammatory sites and its ability to induce inflammatory response. IL-1 will increases the transcription of some APP and decreases the transcription of other hepatic proteins.[5]

IL-6 is a pleiotropic cytokine which involves in regulation of acute-phase response, immune response and haematopoiesis. Acute-phase response induced by administration of endotoxins or septic shock, increased leukaemia inhibitory factors levels in plasma and inflammatory body fluids results in induction of type II APP.

Tumor necrosis factor is considered as a major inflammatory mediator. Effects of tumor necrosis factor on acute-phase induction includes increased biosynthesis of complement proteins factor B and C3 and α1 anti-chymotrypsin. Tumor necrosis factor also decreases the biosynthesis of albumin and transferrin. These three cytokines (IL-1, IL-6 and tumor necrosis factor) can also be carried via the blood to distant sites, inducing an acute-phase reaction.

Glucocorticosteroids which decrease the level of IL-1, tumor necrosis factor, and IL-6 in the peripheral blood via transcriptional and post transcriptional routes and prolong their impact on the target cells through the elevation and the expression of their receptors. Also, prostaglandin inhibit the release of IL-1 from macrophages. There exists apparent feedback mechanisms involving both liver synthesized APP and neuro endocrine factors from the central nervous system, which contribute to regulation of the acute-phase response to inflammation.

SERUM ALBUMIN

The name of albumin protein is taken from Albumen (etymologically goes back to Albus).[6] There exist different types of

albumin, including ovalbumin, human serum albumin (HSA), and bovine serum albumin (BSA). Human serum albumin the most common protein plasma with molecular weight of 66438.

Serum albumin is the most abundant protein, about half of serum protein in plasma, produced by liver. It transports hormones, fatty acids, and other compounds, buffers pH, are among other functions. Albumin is synthesized in the liver as proalbumin, which has an N-terminal peptide which is removed before the nascent protein is released from the rough endoplasmic reticulum.

Human serum albumin research has a long history. It's reported that human serum albumin was precipitated from urine as early as 1500 A.D. Before the 20th century, non-human serum albumin proteins were already being crystallized. Clinical use of human serum albumin occurred as early as the 1940's, when a surgeon by the name of I.S. Ravdin clinically administered purified human serum albumin to seven wounded human patients during the Pearl Harbor attack. Successfully, all seven patients survived. Then in 1992, Xiao He and Daniel Carter solved the three-dimensional atomic structure of human serum albumin using X-ray crystallography to 2.8 angstroms.[7]

FUNCTIONS[8]

a. Acts as the solubilizing agent for long chain fatty acids and is therefore essential for the metabolism of lipids.
b. Binds bilirubin, the breakdown product of haem

c. It binds the therapeutic drugs such as penicillin's, sulfonamides, indole compounds, and benzodiazepines
d. It binds copper(II) and nickel(II) in a specific manner where calcium(II) and zinc(II) in a nonspecific manner and it acts as a transport vehicle for these metal ions in the blood;
e. It is the major protein which is responsible for the colloid osmotic pressure of the blood;
f. When human serum albumin is broken down, the amino acids provide nutrition to peripheral tissue.

Serum albumin is a major protein in human blood plasma which binds water, cations (such as Ca2+, Na+ and K+), fatty acids, hormones, bilirubin, thyroxine (T4) and pharmaceuticals (including barbiturates)

Vitamin D-binding protein which binds to vitamin D and its metabolites, as well as to fatty acids. The isoelectric point of albumin is 4-5.9g/dl.[8]

STRUCTURE

Structurally, the human serum albumin protein is mostly composed of α-helices with an overall structure that resembles a heart shape. Human serum albumin has nine double loops spanning three homologous domains.[9] The domains are named Domain I, II and III. Each domain has two long loops with one shorter loop. The first two loops in each domain are denoted as subdomain A. The remaining loop in each domain forms subdomain B. Thus, human serum albumin has

subdomain IA and IB in Domain I, subdomain IIA and IIB in Domain II and subdomain IIIA and IIIB in Domain III. Subdomains with separate helical structures mediate human serum albumin binding with various endogenous and exogenous ligands. However, while the domains have similar structure, each domain has been shown to have different ligand-binding affinities and functions. Two important binding sites on human serum albumin are Sudlow sites I and II. Sudlow site I located in subdomain IIA and Sudlow site II is located in subdomain IIIA. Sudlow site I has a preferential binding affinity for bulky heterocyclic compounds such as azapropazone, phentylbutazone and warfarin. Sudlow site II seems to preferentially bind to aromatic compounds such as ibuprofen.[10]

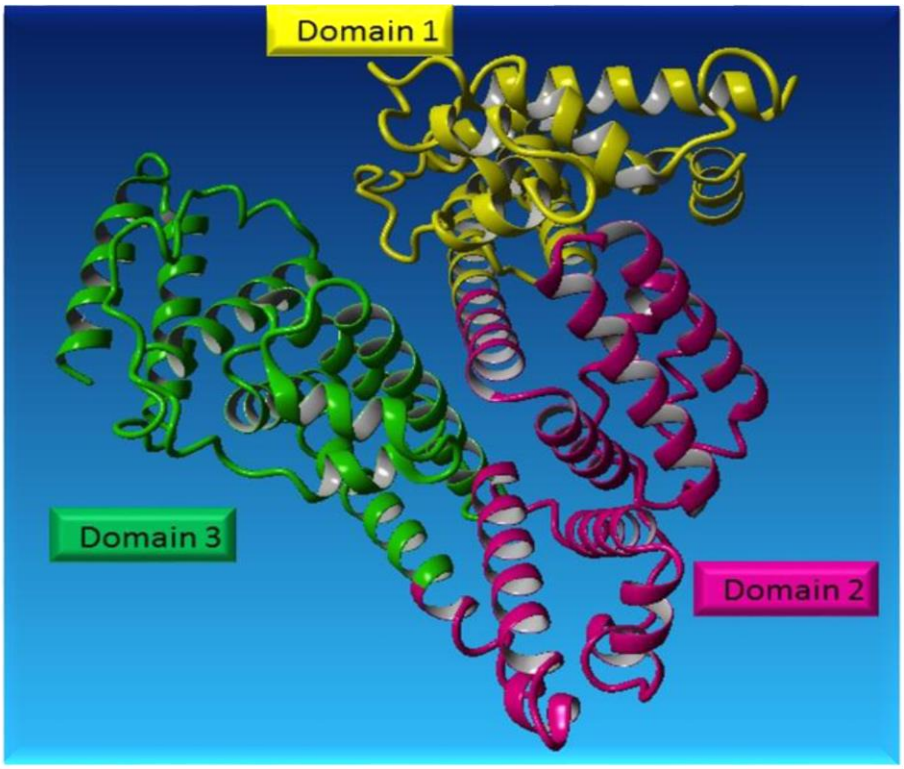


Figure 1. Albumin structure

ENZYMATIC PROPERTIES

Human albumin has an interesting enzymatic properties including: esterase activity, enolase activity, effects on eicosanoids, aryl acylamidase activity, stereospecificity, condensation reactions, binding and activation of drug conjugates. The enzymatic properties of albumin–ligand complexes are as follows: heme and hemin-human albumin, human albumin and Buckminster fullerene, inactivation of reactive oxygen and nitrogen species, metalloenzymes constructed using albumin, lipid peroxide peroxidase activity and nanoparticles.[11]

ALBUMIN SYNTHESIS

A hepatocyte cell are responsible for albumin synthesis but isn't stored by the liver. Once it is produced secreted into the portal circulation. The normal concentration of albumin is 3.5- 5 g/dl in healthy adults and 2.9- 5.5 g/dl in children. 35 percent of the total body albumin exists in the intravascular compartment. The synthesis rate of albumin is approximately 12-25 grams per day. Its biological half-life is approximately 19 days. Routinely albumin turnover occurred around 14 grams in a normal 70 kg adult which is approximately 50 percent in the muscles and skin.[12,13]

Among the factors that modify albumin metabolism and leads to its reduced synthesis, can be noted to decreased gene transcription (such as trauma, sepsis, hepatic diseases, diabetes, decreased growth hormone, decreased corticosteroids) and ribosome disaggregation (like protein depletion, fasting).[14]

Hypoalbuminemia is described as insufficient low levels of Human Serum Albumin (HSA) in the blood and it is a common symptom of ill patients. This symptom is not only a result of reduction in albumin synthesis, but related to the breakdown, protein uptake and leakage to the extra vascular space.[15,16] These are created in diseased conditions such as liver and kidney, infections, AIDS, lymphoma, cancer, poverty nutrition, surgery, burn damage, chemotherapy, spontaneous bacterial peritonitis, taking medication, prematurity infant, acute respiratory distress syndrome, chronic respiratory diseases, acute necrotizing, infant brain hemorrhage, hydrops fetalis, systemic inflammatory response syndrome, sepsis and infant edema.

Table 1: TYPES OF ALBUMIN[17]

Albumin type	definition	M.W (Da)	Pi	aa No.	Applications	Cause of use
OVA	highly functional food protein	47000	4.8	385	• Carrier for drug delivery in food matrix design. • Carrier for controlled drug release.	low cost Availability
HSA	most common protein plasma	66438	5.9	585	• Low blood volume compensation • treatment of related diseases • Drug delivery career	Availability Biodegradability Lack of toxicity

					• Drug and sample stabilization • Cell culture supplement	
BSA	most common protein plasma	69323	4.7	585	• Drug delivery • Usage in pharmaceutical industry	• Medical importance • Abundance • Low cost • Ease of purification • Unusual ligand-binding properties

DRUG INTERACTION

Drug interaction with human serum albumin generally enhances the distribution and bioavailability of the drug depending on the specific pharmacokinetic properties of the drug molecules. Additionally, because of its abundance, human serum albumin plays a significant role in the pharmacokinetic behavior of a variety of drugs, including: drug half-life in the bloodstream, regulating drug efficacy, decreasing drug toxicity, and improving drug targeting specificity.[18]

APPLICATIONS

Serum albumin is utilized under various clinical conditions. It restores blood volume, emergency treatment of shock, acute

management of burns, and other situations associated with hypovolemia are some of the clinical applications of albumin.

Serum albumin is a important biomarker for liver function synthesis, and also for several diseases, such as, inflammatory disorders, brain tumors, rheumatoid arthritis, myocardial ischemia, cancer, blood brain barrier, renal disease, cerebrovascular disease, cardiovascular risk disease, and also in disorders which requires the blood sugar control. In addition, albumin has different applications in the related research fields such as, cryopreservation, stabilizer of some of the proteins and as a supplement in cell culture. Novel application of HSA is consisted of a fusion of peptides, a drug nanocarrier and an oxygen transporter.[17]

POST-TRANSLATIONAL GLYCATION

Human serum albumin can undergo post-translational glycation. Glycation is the binding of a protein with a sugar molecule without the assistance of an enzyme. On human serum albumin, these sites include Arginine 114, 218 and 428 as well as Lysine 186.

Glycated human serum albumin has been shown to have five-fold increase in binding activity to L-tryptophan compared to non-glycated human serum albumin. While increased glycation of human serum albumin is usually correlated with increased blood glucose levels, as seen in diabetic patients, a recent *in vitro* study suggests that Zinc concentrations may also regulate human serum albumin glycation.[19]

Glycated human serum albumin stays in the blood stream for 2-3 weeks. Therefore, tools measuring glycated human serum albumin may function as better monitors for glycemic levels over longer periods of time.

Human serum albumin can also undergo cysteinylation, or the addition of another cysteine to Cysteine-34 on human serum albumin via a disulfide bond. This modification has been suggested to be facilitated by cystathionine β-synthase (CBS), as CBS deficient mice lack cysteinylated human serum albumin.This modification was found in patients with liver and kidney diseases, as well as patients with diabetes. Cysteinylated human serum albumin levels correlate with high-risk pregnancies and uteroplacental insufficiency (UPI), suggesting that measuring cysteinlyated human serum albumin levels may be advantageous to monitor pregnancies affected by UPI. Increased human serum albumin cysteinylation has also been observed at the end stage of renal disease patients.[20]

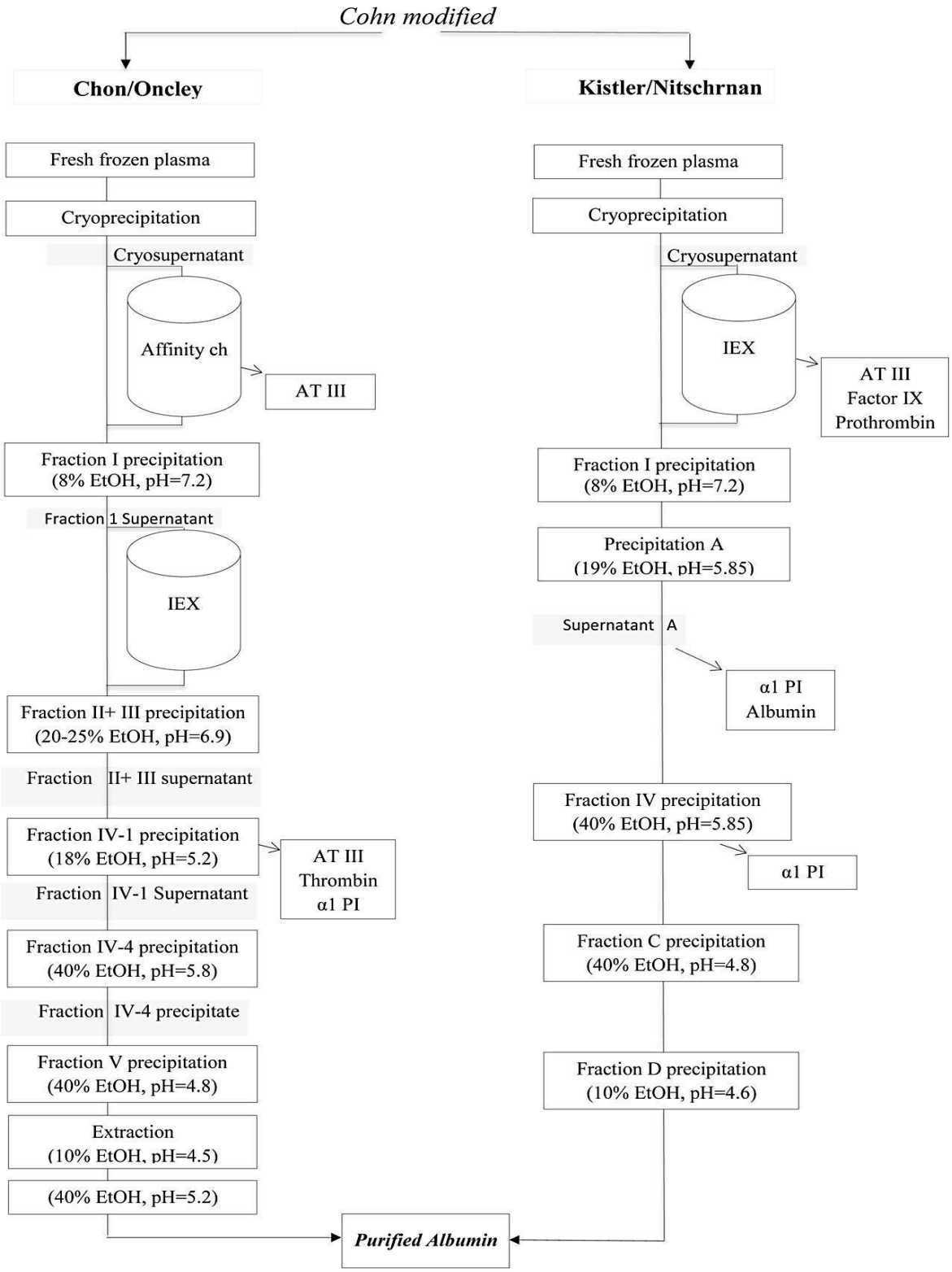
Some new post-translational modifications of human serum albumin include S-guanylation and dehydroalanine conversion. *S*-guanylation of cysteine 34 is a recently reported modification of human serum albumin discovered when comparing blood samples between healthy patients and hemodialysis patients. *S*-guanylation modification occurs when an 8-nitroguanosine 3′,5′-cyclic monophosphate group reacts with sulfydryl groups of human serum albumin. While it is unclear how this modification may affect drug binding, research

suggests that this protein may function as a endogenous antibacterial agent. Only minor structural conformational changes were observed with this modification.[7]

HUMAN ALBUMIN PURIFICATION METHODS [17]

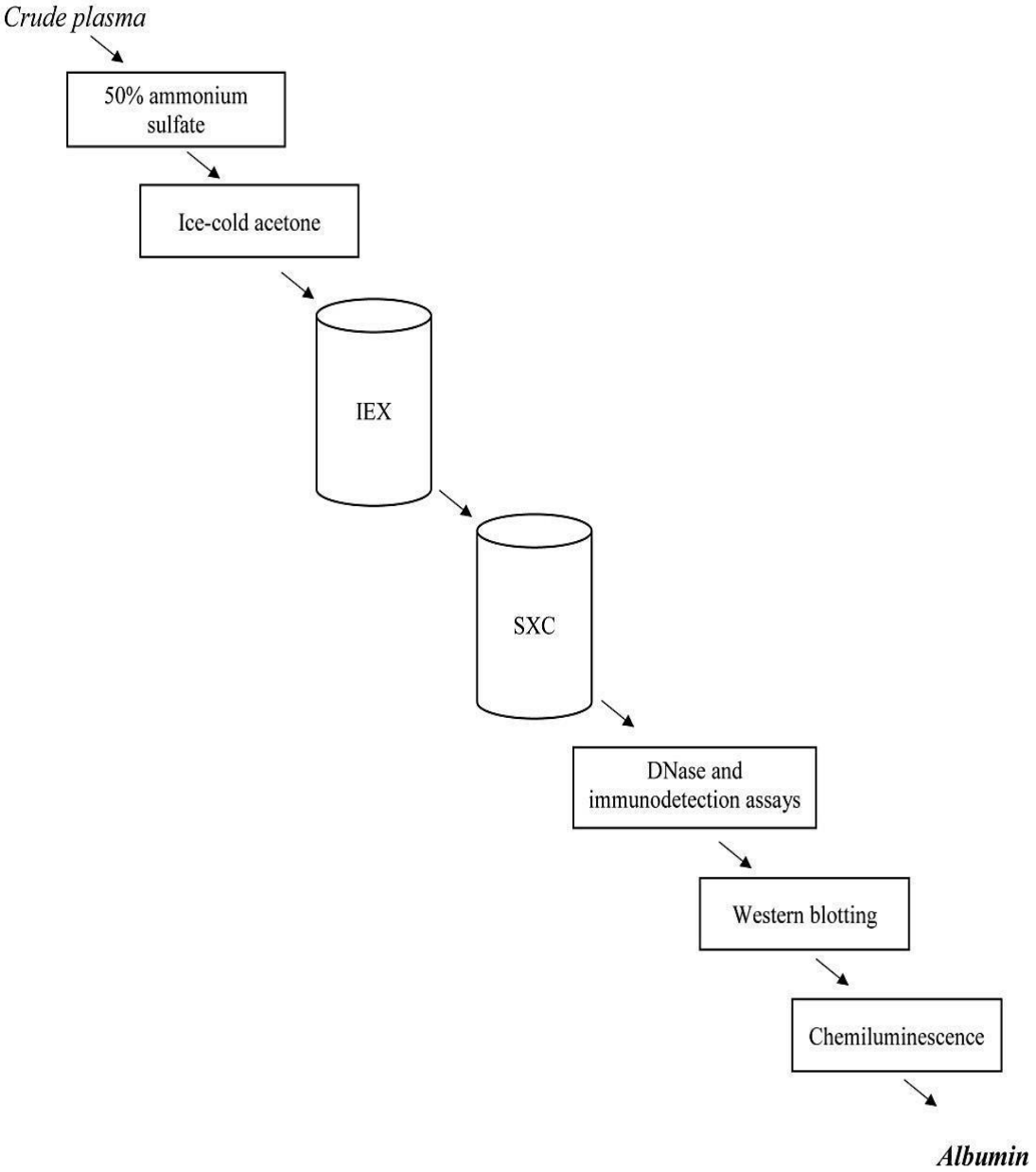
- Plasma fractionation method with ethyl alcohol
- The Cohn method combined with chromatography
- The Cohn method combined with liquid chromatography
- Purification from placenta
- Heat shock method
- Ammonium sulfate precipitation combined with liquid chromatography
- TCA/Acetone precipitation method
- Column chromatography for the purification of HAS
- Ion exchange chromatography (IEC)
- Simulated moving bed chromatography (SMB)
- Steric exclusion chromatography (SXC)
- Expanded bed adsorption chromatography
- Affinity Chromatography
- Dye ligand affinity chromatography
- Immobilized Metal-ion Affinity Chromatography (IMAC)
- Boronate Affinity Chromatography
- Immunoaffinity chromatography (IAC)

Cohn method combined with chromatographic techniques for purification of albumin



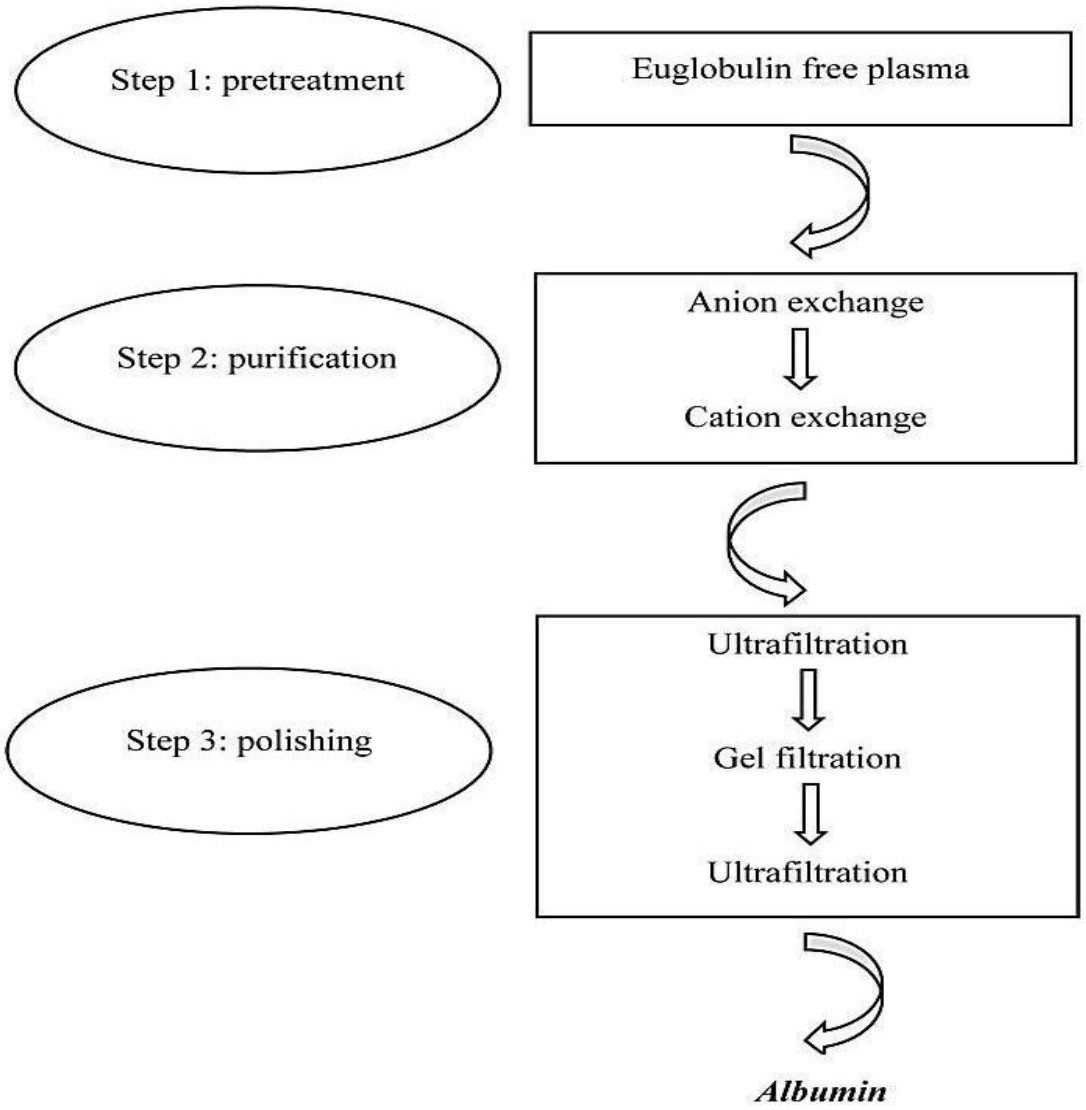
AT III: Antithrombin III, **Ch**: Chromatography, **IEC**: Ion exchange chromatography, **α1-PI**: Alpha1-Proteinase Inhibitor

Ammonium Sulfate Precipitation Combined With Liquid Chromatography



EC: Ion exchange chromatography, **SXC**: Steric exclusion chromatography

Purification and Polishing Methods for Human Serum Albumin (HSA).



DIAGNOSTIC SIGNIFICANCE OF SERUM ALBUMIN

Serum albumin levels might be a practical marker of the general health status as they describe the severity of an underlying disease and mortality in elderly. Reduced serum albumin levels in many conditions such as inflammatory states, liver disease and renal diseases. Moreover, malnutrition also may be monitored by means of the serum albumin concentration **(Don &kaysen2004)**[21]. It can be suggested that lower albumin levels have a complex etiology rather than reduced

protein intake alone contributing to hypoalbuminaemia. **Rigaud et al 2000**[22]

Periodontitis has been implicated as a risk factor for medical disease such as cardiovascular disease and diabetes mellitus (Tylor 2001)[23]

Yoshihara et al. (2003)[24] have recently reported that the number of untreated teeth was a significant factor associated with serum albumin concentration in elderly. Hence, it is suggestive that oral disease burden might be monitored by the levels of serum albumin. However, few studies have been performed or reported on the relationship between periodontal disease status and serum albumin concentration.

REVIEW OF LITERATURE

Corti MC *et al., (1994)*[25] Investigated the relationship between serum albumin level and all-cause mortality in an elderly population aged 71 years and graded increases in mortality rate with decreasing albumin level/while hypoalbuminemia was associated with increased mortality rate. It is apparent that oral disease might be indicated and monitored by levels of serum albumin.

Grenier D *et al., (2001)*[26] Investigated the role of Gingipains in growth of *Porphyromonas gingivalis* in the presence of Human Serum Albumin and he stated although all of the proteins were degraded by *P.gingivalis*, only human serum albumin and transferrin supported growth during serial transfers in a chemically defined medium (CDM). Gingival crevicular fluid samples from diseased periodontal sites contained low-molecular-mass albumin fragments, whereas samples from healthy sites did not.

Yoshihara *et al., (2003)*[24] Evaluated the association between serum albumin concentrations and root caries in community dwelling older individual. 763 individuals (600 70-year-olds and 163 80-year-olds) were selected randomly. The variables body composition, blood measurements, daily nutrient intakes, and root caries were measured and relationship between root caries and serum albumin concentration was evaluated. Results showed serum albumin concentrations between subjects with untreated root caries (DT = 0 and DT > 3) were 75.56

mg/dl in 70-year-olds and 202.97 mg/dl in 80-year-olds ($p < 0.05$) and suggested that a relationship exist between root caries and serum albumin concentration in elderly subjects.

Shaper AG *et al., (2004)*[27] Stated that the low albumin blood levels, even low normal, correlate with increased risk of death from cardiovascular disease (CVD) such as coronary heart disease and strokes. This correlation was studied by smoking cigarettes followed by 7,690 British men from age 40 to 59 for an average of 16.8 years. Results showed the low serum albumin level strongly related to cigarette smoking. The correlation between albumin level and CVD was only seen in current or previous smokers. Albumin was the lowest in the heaviest smokers and concluded the albumin may be a marker for the CV effects of smoking cigarettes.

Don BR *et al., (2004)*[21] Reviewed the serum albumin relationship with inflammation and nutrition. Hypoalbuminemia is the result of combined effects of inflammation and inadequate protein intake in patients with chronic disease such as chronic renal failure. Hypoalbuminemia is a strong predictor of mortality in patients with chronic renal failure, and the major cause of death in this population is due to cardiovascular events. Inflammation associated with vascular disease and causes injury to the vascular endothelium, and hypoalbuminemia as two separate expressions of the inflammatory process. Albumin is a myriad of important physiologic effects that are essential for normal health.

Ibrahem HM *et al.*, (2006)[28] Assess the plasma antioxidant status in patients with periodontitis using serum albumin concentration as a criterion index.20 individuals were included (10 healthy control and 10 with chronic periodontitis). Serum albumin level was detected by the bromocresol green albumin (BCG) colorimetric method. They stated that Patients with periodontitis had a significant decrease in the level of serum albumin than that of the healthy subjects ($p<0.05$).Concluded decreased level of serum albumin in patients with periodontitis, which indicates decreased antioxidant activity in patients with periodontitis on comparison with the healthy individuals.

Ogawa H *et al., (2006)*[29] Evaluated the relationship between the periodontal disease and general health status in community dwelling elderly using serum albumin concentration as a criterion index of the severity of an underlying disease and nutrition status. Serum albumin level was detected by the bromocresol green albumin method. 368 subjects were included, clinical attachment levels at six sites of all teeth were measured and concluded that there might be an inverse relationship between periodontal disease.

Kshirsagar AV *et al., (2007)*[30] Evaluated the relationship between periodontitis and two measures of systemic inflammation, serum albumin and C-reactive protein (CRP), were examined among patients who were receiving chronic outpatient haemodialysis. A total of 154 patients completed the study. The mean age was 54.6 yr (SD 13.3), and average duration of dialysis was 4.0 yr (3 mo to 16 yr). Common

causes of end-stage kidney disease are hypertension (12.3%), diabetes (22.1%), glomerulonephritis (7.1%), and other (58.4%).The average number of teeth was 20.3 (SD 8.4). Thirty-five (23%) patients had periodontitis cases. Severe periodontitis were associated with low serum albumin level (odds ratio 8.20; 95% confidence interval 1.61 to 41.82; *P*-0.01) compared with individuals without severe periodontitis.

Iwasaki *et al., (2008)*[4] Evaluated the relationship between periodontal disease and general health status in community dwelling elderly using the serum albumin concentration as a criterion index of the severity of an underlying disease and nutrition.600 subjects included in this study. Clinical attachment level at six sites of all teeth present were measured. Serum albumin concentration at baseline ranged from 3.4 – 5.0g/dl with a mean of 4.3±0.2. Stated that serum albumin concentrations had a significant effect on periodontal disease progression among non smokers and concluded that serum albumin concentration is a significant risk predictor for periodontal disease progression among elderly non smokers.

Shi D *et al., (2008*)[31] Explored the characteristics of peripheral blood cellular and serum protein parameters in patients with Aggressive Periodontitis. Clinical parameters including probing depth, clinical attachment loss were examined blood cell variables, including leukocyte, neutrophil and lymphocytes counts were noted as serum protein parameters including total protein, albumin, globulin and albumin globulin ratio, were analyzed. Results showed albumin level

and albumin globulin ratio were lower in Aggressive Periodontitis group than in the control group(47.65±2.45g/dl versus 48.88±2.13) and concluded that the patients with Aggressive Periodontitis may have elevated peripheral leukocyte numbers and serum globulin levels as well as decreased serum albumin and albumin /globulin ratios compared to controls. Concluded that these changes might be associated with the severity of periodontal destruction

Kolte RA *et al.*, (2010)[32] Evaluated the relationship between periodontal disease and general health status in adults using the serum albumin concentration. Totally 100 patients of both genders with age range of 40 to 70 years were included in the study. Patients were divided into two groups: Group I periodontally healthy subjects and Group II patients with periodontitis. Results showed The mean serum albumin levels for Group I was 4.47 g/dl with standard deviation (SD) of 0.276 and for Group II, the mean value of serum albumin was 4.61 g/dl with SD of 0.273and suggested that there is an inverted relationship between serum albumin concentration level and chronic periodontal disease.

Maruyama T *et al., (2012)*[33] Conducted a study among 170 people of head and neck cancer patients and found an association between periodontal disease and serum albumin concentration However, there were no significant differences in serum parameters between the healthy and test groups.

Amitha R *et al., (2012)*[34] Evaluated the association between serum albumin concentration and periodontitis. Total of 60 subjects were included and divided into two groups, Group A -30 subjects with healthy periodontium Group –B 30 subjects with chronic periodontitis with clinical attachment loss >4mm in greater than 30% of the sites examined, aged between 30 – 40 years and concluded that there is a inverse association between serum albumin concentration and loss of attachment in periodontitis patients and the reduction in serum albumin concentration in periodontitis group when compared to the healthy controls.

Siribamrungwong M *et al.*, (2012)[35] Evaluated the effect of periodontal treatment in maintenance haemodialysis patients. Periodontal diseases were evaluated in 30 stable maintenance haemodialysis patients by using clinical periodontal status by plaque index (PI) and periodontal disease index (PDI). Hematological, biochemical, nutritional, and dialysis-related parameters as well as highly sensitive C-reactive protein (hs-CRP), a sensitive systemic inflammatory marker, were analyzed before and after periodontal therapy. He stated that maintenance hemodialysis patients had high prevalence of periodontal disease (63%). Baseline, highly sensitive CRP positively correlated with clinical periodontal status (PI, r = 0.74, p<0.001; PDI, r = 0.66, p<0.001), but negatively correlated with hemoglobin (r = −0.51, p <0.001), serum albumin (r= −0.61, p= 0.002), After completion of periodontal therapy , the PI and PDI significantly declined from 2.13 to 1.48 (p = 0.001) and 3.53 to 2.52 (p = 0.001),

respectively, while hs-CRP significantly declined from 3.8 to 0.6 mg/L (p <0.001)and serum albumin level increased from 3.15 to 3.38 mg/dL (p = 0.003), reflecting improved nutritional status of the patients after periodontal treatment. He concluded that periodontitis is an important source of chronic inflammation. Treating the periodontal diseases can improve systemic inflammation, nutritional status, and erythropoietin responsiveness in the haemodialysis population.

Saravanan AV *et al., (2012)*[36] Evaluated the association between serum albumin and Chronic Periodontitis in elderly individuals. 60 systemically healthy individual included in this study. They were divided into control and experimental groups of 30 each. Plaque index, Gingival index, Probing depth, Clinical attachment level were recorded. In experimental group serum albumin concentration ranged from 2.2 -3.6 g/dl. The mean serum albumin levels were significantly lower in subjects with 6mm and above of clinical attachment loss as compared to controls and concluded that there is an association between serum albumin and chronic periodontitis.

Akpinar EE *et al., (2013)*[37] Evaluated the role of Albumin level and Blood Urea Nitrogen/ Albumin Ratio in Prediction of Prognosis of Community Acquired Pneumonia .Total of 216 patients were enrolled. He stated that Low albumin level is a independent predictive factor for the development of complications (OR: 4.902, 95% CI: 1.595 to 14.925, p=0.005).Concluded the Low albumin level is a more important predictor than BUN/Alb ratio for prognosis of CAP.

Cerasela S *et al., (2013)*[38] Evaluated the interrelation between chronic periodontitis and chronic renal disease by quantifying the glomerular filtration rate markers. Sixty patients with incipient chronic renal disease were included in this study; they were divided in two groups (30 patients with periodontal disease and 30 patients without periodontal disease). They measured serum and urinary levels of renal function markers (urea, Creatinine, albumin); glomerular filtration rate was estimated from the Creatinine clearance and the changes of the albumin (mg)/Creatinine (g) ratio were evaluated in the 24 hours urine sample. Results showed a great number of sites with probing depth between 3 and 5mm were correlated to higher Creatinine elimination rates. A high number of sites with probing depth<3mm and low bleeding on probing was associated with higher levels for Creatinine, Creatinine clearance and low levels of serum albumin. He concluded that the periodontitis patients presented modifications for serum or urinary markers of renal dysfunctions, suggesting that periodontitis and periodontal treatment might influence the renal function in certain conditions.

Wahid A *et al., (2013)*[39] Reviewed about Bidirectional Relationship between Chronic Kidney Disease & Periodontal Disease. They stated that Periodontitis increases systemic inflammatory burden leading to worsening of CKD which in turn has been found to be negatively affect CKD of patients on haemodialysis therapy by altering their serum albumin and C-reactive protein levels. As Hypoalbuminemia leads to increased mortality in CKD patients and concluded albumin is a strong

prognostic marker in ESRD patients on HD therapy, it needs to be as an outcome measure in clinical trials evaluating the effect of periodontal treatment on impairing quality of life of CKD patients.

Pimpale S *et al., (2014)*[40] Evaluated the correlation between periodontal disease and serum albumin concentration in chronic periodontitis patients.400 subjects divided into two age groups between 25 to 35 and 35 to 45 years (200 in each group, comprising 100 healthy and 100 chronic periodontitis patients). Level of serum albumin was compared between the patients affected with chronic generalised periodontitis & healthy individuals. Statistical analysis using Student's unpaired t-test was employed to compare the difference between the two means. In both age groups, the difference in healthy and chronic periodontitis patients in terms of serum albumin level was found to be statistically significant ($p < 0.05$) and stated that the serum albumin levels in chronic periodontitis patients had decreased in comparison with healthy individuals of the same age group. Concluded serum albumin concentration could be a significant risk indicator for patients with chronic periodontitis.

Rodrigues VP *et al., (2014)*[41] Investigated the association between periodontal status and serum biomarkers levels in haemodialysis patients. Total of 96 haemodialysis patients were included. The subject was diagnosed with periodontitis at least two inter-proximal sites in different teeth with CAL ≥4 mm and/or at least two inter-proximal sites in separate teeth with PD ≥5 mm. Biochemical and haematological

parameters are serum albumin, phosphorus, creatinine, transferrin, ferritin, iron, alkaline phosphatase, calcium, potassium and hemoglobin were collected. Stated that there was a positive association of periodontitis with hypoalbuminaemia (OR = 9.10, p = 0.006) and he concluded that there is a association between periodontitis with serum albumin levels in haemodialysis patients.

Kaur N *et al., (2015)*[42] Conducted a Study to evaluate the relationship between the periodontal health status and serum albumin levels, total of 60 subjects with age range of 40-70 years were divided into two groups in this study. Group I Clinically healthy subjects and Group II patients with Chronic Periodontitis, patients. The results showed mean serum albumin levels for group I was 4.815 g/dl with standard deviation of 0.127 and for group II the mean serum albumin levels was 4.219 g/dl with standard deviation 0.174 and stated that the difference between serum albumin levels in group I and group II were found to be statistically significant ($p \leq 0.001$) and concluded that there is an inverse relationship between the serum albumin concentration and chronic periodontal disease.

Rajesh K.S *et al., (2015)*[43] Determined the levels of WBC's, Platelets and Serum protein including Total Protein ,Serum Albumin, Serum Globulin in subjects with Chronic Periodontitis and compared to healthy controls. Total of 50 systemically healthy subjects were included in this study and he concluded that difference in values of hematological parameters between subjects with clinically healthy

gingiva and subjects with chronic periodontitis, was found to be statistically significant.

Byrne DP *et al.*, *(2015)*[44] Evaluated breakdown of albumin and haemalbumin by the cysteine protease interpain A, an albuminase of Prevotella intermedia. Breakdown of albumin was examined over a range of pH and in the presence of reducing agent; conditions which prevail in sub- and supra-gingival plaque. Interpein A digested haemalbumin more efficiently than apoalbumin, especially under reducing conditions at pH 7.5. Under these conditions InpA was able to substantially degrade the albumin component of whole human plasma and concluded that The data point to InpA as an efficient "albuminase" and able to degrade the minor fraction of haem-bound albumin in plasma. InpA may contribute significantly to haem acquisition by P. intermedia under conditions of low redox potential and higher pH in the inflammed gingival crevice and diseased periodontal pocket where haem availability is tightly controlled by the host.

Pereira GR*et al.*, (2015)[45] Evaluated the predictability of early changes in serum albumin (sAlb) on the two-year mortality of incident hemodialysis patients. 1,679 incident patients were included. He stated that 923 patients had low sAlb which is $\leq$ 38 g/L (Low sAlb Group) and 756 ones had sAlb > 38.0 g/L (Adequate sAlb Group).Concluded early sAlb changes showed a significant predictive power on mortality at 2 years in incident haemodialysis patients.

Veisa GA*et al.*, (2016)[46] Evaluated the relationship between albumin level, malnutrition and periodontal status in a haemodialysis patients. They analysed the inflammatory status and malnutrition in 200 haemodialysis patients, mean age 54 ± 14 years. At baseline evaluated: a. nutritional status assessed by anthropometric measures- post dialysis body weight (BW), body mass index (BMI); b. subjective global assessment score (SGA); c. biochemical parameters c-reactive protein (CRP), TNF-α**,** pre-dialysis serum albumin, IL-6 and assessment of periodontal disease status. The patients were followed-up for 2 years. malnutrition is linked with age (albumin, SGA, BMI) and CRP (SGA, albumin) and he stated albumin and nutritional status(evaluated by SGA score) were associated with a significantly increased death risk.

Reshama Y *et al.*, (2016)[47] Evaluated the serum albumin and other biochemical Parameters in patients with chronic periodontitis. Total of 80 subjects included, 40 were chronic periodontitis patients and 40 were healthy subjects. The subjects in the study and control group were aged between 30 to 60 years. The biochemical parameters which were evaluated were serum albumin, serum al alaninetransaminase, aspartate transaminase and alkaline phosphatase. It was found that serum albumin levels were significantly lower in chronic periodontitis patients than healthy subjects and concluded that there is inverse association was seen between serum albumin and clinical attachment loss in chronic periodontitis patients. Serum ALT and ALP showed significant increase in patients with chronic periodontitis as compared to healthy subjects.

Surya V *et al., (2016)*[48] Compared the Serum Albumin & Serum A/G ratio in normal healthy adults, oral leukoplakia and oral cancer total of 100 subjects included categorized as healthy (Group I = 20), oral leukoplakia (Group II = 40) & Oral squamous cell carcinoma (Group III = 40).He stated that gradual decrease in serum albumin level in leukoplakia patients compared to normal healthy control and then further in oral cancer patients. There is a significant reduction in Serum A/G ratio in oral cancer patients when compared to leukoplakia patients. He concluded that there is a significant decrease in serum Albumin levels and serum A/G ratio in oral carcinoma.

Takeshi *et al.,* (2016)[49] Evaluated the association between dental health and nutritional status in chronic obstructive pulmonary disease**.** Periodontal status were assessed using bleeding on probing (BOP), pocket depth (PD), and plaque–control ratio (PCR). Nutritional status were assessed by using body mass index, lean body mass, and serum albumin levels. Stated That COPD group (n ¼ 60) had fewer remaining teeth, greater BOP, greater PD, and lower serum albumin levels compared with smokers without COPD (n ¼ 41) and nonsmokers (n ¼ 35; p<0.001). COPD was an independent risk factor for poor periodontal health, demonstrated by fewer remaining teeth (relative risk (RR), 5.48; p ¼ 0.0024), BOP (RR, 12.8; p ¼ 0.0009), and having >30% of remaining teeth with a PD _ 4 mm (RR, 4.82; p ¼ 0.011). A significant negative correlation existed between the number of teeth with a PD _ 4 mm and serum albumin level (r2 ¼ 0.127; p ¼ 0.013). Concluded that poor periodontal health was associated with

hypoalbuminemia, suggesting poor nutritional status and inflammation in COPD.

Maruyama T *et al.*, (2017)[50] Evaluated the Association between periodontitis and prognosis of pancreatobiliary tract cancer. Totally 77 patients were diagnosed with primary cancer of the pancreas, bile ducts or gallbladder. And results showed The serum CRP concentration was significantly higher and the serum albumin concentration was significantly lower in patients with severe periodontitis group when compared to those without severe periodontitis ($P<0.05$) and suggests that periodontitis affects the prognosis of pancreatobiliary tract cancer by decreasing serum albumin level.

Brahambhatt nilam *et al., (2018)*[51] Conducted a study to evaluate the relationship between periodontal health status and serum albumin levels. Total of 100 subjects included in this study. Patients were divided into two groups. Group I clinically healthy subjects and group II patients with chronic periodontitis and loss of attachment ≥5mm.serum albumin concentration was estimated by bromocresol green albumin method. Results showed mean value of serum albumin level for group I was 4.710g/dl with standard deviation of 0.127 and for group II the mean value of serum albumin level was 4.125g/dl with standard deviation of 0.128. The difference between serum albumin level in group I and group II were found to be statistically significant and concluded that there is a inverse relationship and statistically

significant correlation between the serum albumin concentration and periodontal disease.

Tsai MH *et al., (2018)*[52] Determined the prognostic value of the serum albumin level among patients with advanced head and neck squamous cell carcinoma (HNSCC) undergoing surgery with simultaneous free flap reconstruction. A total of 233 patients with advanced head and neck cancer undergoing tumor resection and immediate microvascular free flap reconstruction in a tertiary referral center were enrolled. Results multiple regression analysis showed a higher risk of postoperative major wound infection among patients with postoperative hypoalbuminemia than in their counterparts (odds ratio [OR] 9.811, 95% CI [2.288-42.065], (p;0:002) Concluded that Postoperative hypoalbuminemia is a useful indicator for the development of postoperative complications.In addition, preoperative hypoalbuminemia is a negative prognostic factor for patients who have undergone tumor excision and free flap reconstruction for the advanced stage of HNSCC.

Ausavarungnirun R *et al.*, (2018)[53] Evaluated association of severe periodontitis and hypoalbuminemia in chronic kidney disease patients. Totally 29 patients with different stages of CKD were included. Ninety-eight (76%) were men. The age range was between 30-86 years, with an average age of 61 ± 11 years and stated that The levels of serum albumin decreased when eGFRs were a decline (γ=0.33; P=0.002). Severe periodontitis, eGFR lower than 30 ml/min/1.73m2 and brushing teeth (>1time/day) associated with hypoalbuminemia

(defined as <3.8 g/dL) [odds ratio (and 95% confidence interval) of 5.88 (1.64-21.11), 5.80 (1.58-21.35) and 0.16 (0.05-0.60)], respectively. Concluded that accounting for the immunity of CKD patients, dental diseases, periodontal diseases, and oral mucosal diseases are of significant concern. Routine dental examinations and proper preventive dental care are suggested in CKD patients, especially in the early stages of CKD.

MATERIALS AND METHOD

This study was conducted at the Department of Periodontology, Adhiparasakthi Dental College and Hospital, Melmaruvathur (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. Ethical clearance for the study was obtained from institutional review board (Reference No : 2016-MDS-BrII-VID-05/APDCH)

STUDY DISGN

A total number of 60 subjects were enrolled in the study with the age range of 18 -50 years from the outpatient division of APDCH. 60 subjects were divided into three groups. Group- 1 consists of 20 healthy control subjects, Group-II consists of 20 generalised chronic periodontitis patients, Group-III consists of 20 localized aggressive periodontitis. Patients were selected according to in each group fulfilling following criteria.

METHOD

Total of 60 subjects were divided into 3 groups

GROUP I – Healthy controls -20

GROUP II – Chronic generalised peridontitis -20

GROUP III – Localized aggressive periodontitis -20

INCLUSION CRITERIA

GROUP I

Clinically healthy gingiva

Absence of loss of attachment

Bleeding on probing involving <20%

GROUP II

Subjects having with Clinical attachment loss of ≥3mm in more than 30% of the sites

Bleeding on probing in more than 30% of the sites

GROUP III

Localized first molar or incisor presentation with interproximal attachment loss on atleast two permanent tooth one of which is first molar and incisors and involving no more than two teeth other than first molar and incisor.

EXCLUSION CRITERIA

Any systemic diseases

Smokers

Pregnancy and lactation

Has undergone any periodontal therapy for the past 1 year

Following clinical index and periodontal parameters were recorded

Bleeding index (Ainamo & Bay)

Probing pocket depth

Clinical attachment level

GINGIVAL BLEEDING INDEX: (AINAMO AND BAY)[54]

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 in a percentage by calculating in the following measures,

$$\frac{\text{No of bleeding sites on probing}}{\text{Total numbers of sites probed}} \times 100$$

PROBING POCKET DEPTH[55]

Pocket depth refers to the depth measured 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 contact points. Facial and oral pockets were measured at the midline of the roots. Buccal and lingual pockets of multirooted teeth were measured at the mesial roots in order to avoid 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. A probe reading that falls between two calibrated marks on the probe should be rounded upward to the highest millimetre. Out of 6 surfaces per tooth, the highest probing depth value is taken as the probing depth of that individual tooth.

CLINICAL ATTACHMENT LEVEL[55]

Level of attachment refers to the distance between the base of the pocket and CEJ. The loss of attachment was assessed on the 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 gingival had been subject to recession and the CEJ was exposed, the loss of attachment equaled 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 1mm or less was recorded as 1mm, measurements exceeding 1mm but less than 2mm were recorded as 2mm.

BLOOD PARAMETER

Serum albumin

METHODS TO BE FOLLOWED

The patients were diagnosed with healthy gingiva, chronic generalised periodontitis and localized aggressive periodontitis who reported to the Department of Periodontology and expressed willingness to participate in the study were recruited. Before undergoing the examination a written informed consent was obtained from all the study participants and they were subjected to the measurement of clinical index that includes gingival bleeding index and clinical parameters including probing pocket depth and clinical attachment level.

BLOOD SAMPLE COLLECTION

- ✓ Under aseptic condition 2ml of venous blood sample will be collected from the antecubital vein and transferred into the vial
- ✓ Collected blood sample centrifuged at 2,500 rpm for 10 min using a centrifugal machine.
- ✓ Biochemical value of serum albumin level were measured by bromocresol green albumin method using a biochemical analyzer.

ESTIMATION OF SERUM ALBUMIN

Principle

Bromocresol green in solution has two forms,monovalent yellow form with undissociated phenol group and a divalent blue form. Albumin combines with monovalent yellow form upsetting the balance and a blue colour is produced and is proportional to the concentration of albumin.[56]

Albumin in the sample reacts with bromocresol green in acid medium forming coloured complex that can be measured by spectrophotometry.

BCG + Albumin PH 4.3 BCG-albumin complex

COMPOSITION

Reagent 10 × 60 ml

Acetate buffer 100 mmol/l

Bromocresol green 0.27mmol/l

Detergent, PH 4.1

STORAGE AND STABILITY

Reagent Store at 2-8 °C.

Reagents is stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

Reagent presence of particulate material, turbidity, absorbance of the blank over 0.200 at 630 nm (1 cm cuvette).

REAGENT PREPARATION [57]

The Reagents are provided ready to use.

Opened reagents and stored in the analyzer chamber at 2- 8˚C are stable 2 months.

AUXILIARY REAGENTS

Biochemistry calibrator (biosystems.cod.18011) or biochemistry calibrator human (biosystems.cod.18044)

SAMPLES

Serum or plasma (EDTA, citrate or heparine) collected by standard procedures.

Albumin in serum and is stable for 3 days at 2-8˚C..

REFERENCE VALUES[57]

Serum

Newborn 2-4 days - 28-44 g/dl

4 days to 14 years - 38-54 g/dl

Adult - 35-50g/dl

>60 years 34-48 g/dl

CALIBRATION

It is recommended to do reagent blank every day and calibration at least 2 months, after reagent lot change or as required by quality control procedures.

PROCEDURE

ASSAY parameter

Wavelength ……640±30 nm

Temperature ……37˚C

Analysis mode……end point

TUBES	Blank	Standard
Sample/Standard	-	3µl
Distilled water	3 µl	_
Reagent	300µl	300 µl

Mix and incubate for 2 minutes and read adsorbance.

Metrological characteristics

Metrological characteristics have been obtained using a BA400 analyzer.

- ✓ detection limit : 1.1 g/dl
- ✓ linearity limit : 70g/dl
- ✓ repeatability (within run):

Mean concentration	CV	n
25.7 g/dl	1.8%	20
39.8g/dl	1.5%	20

Reproducibility (run to run):

Mean concentration	CV	n
25.7 g/dl	2.8%	25
39.8g/dl	2.0%	25

CALCULATIONS

$$\frac{\text{(A) Sample}}{\text{(A) Standard}} \times 5 \text{ (Standard conc)} = \text{g/dL albumin}$$

BASIC ARMAMENTARIUM

1. Mouth mirror
2. Unc 15 probe
3. Surgical gloves
4. Mouth masks
5. Tweezers
6. Cotton rolls
7. 2ml blood collection sample tubes
8. Albumin kit

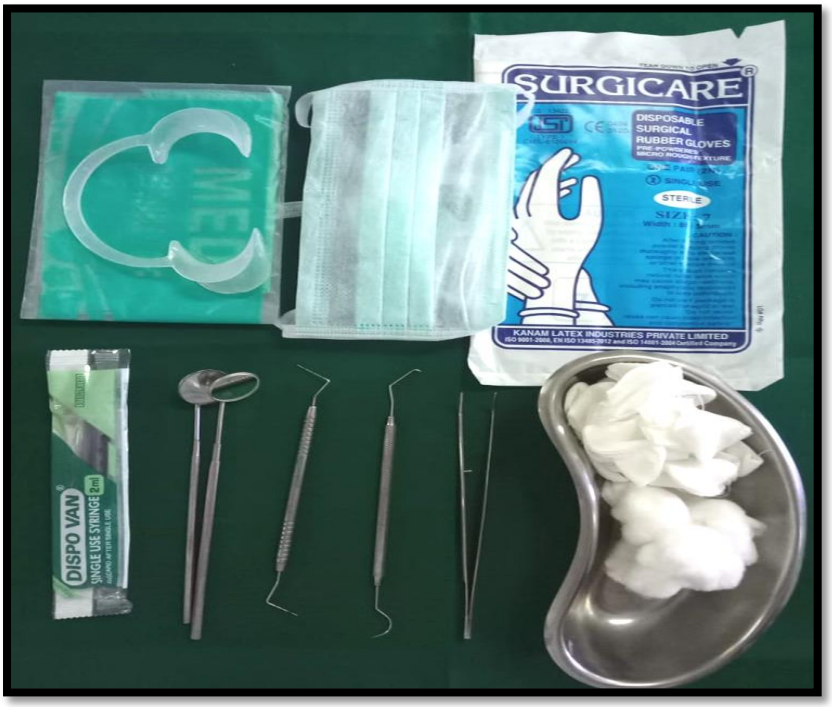


Figure 2: Basic armamentarium

Figure 3: Chronic generalised periodontitis

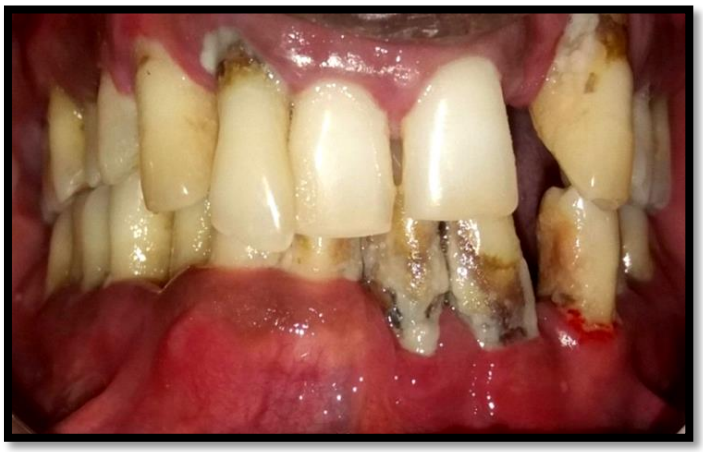


Figure 3a : Facial view

Figure 3b : Posterior view (right side)

Figure 3c : Posterior view (left side)

Figure 3d: Probing depth>5mm

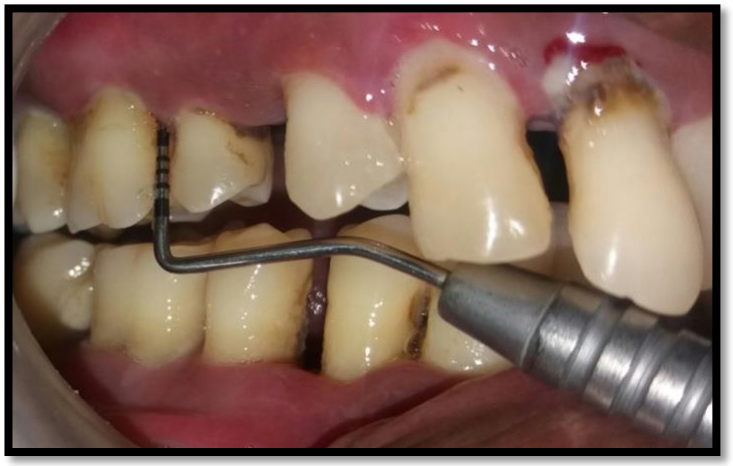


Figure 3e: Probing depth>6mm

Figure 3f: Probing depth >8mm

Figure 4: Localized aggressive periodontitis

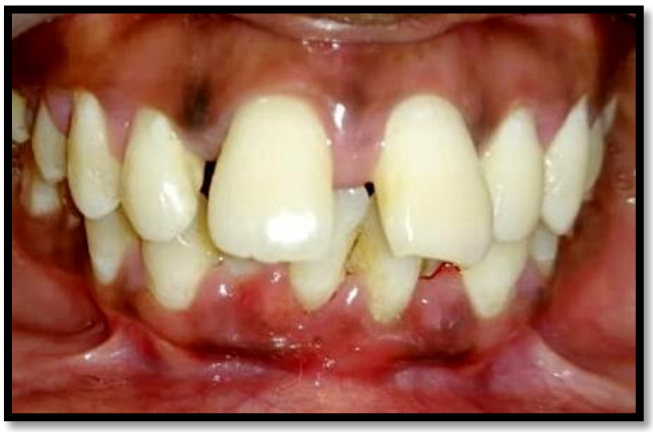


Figure 4a: Facial view

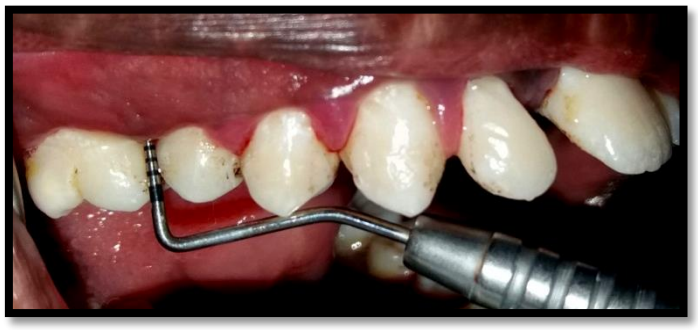


Figure 4b: Probing depth >10mm

Figure 5 : Collection of blood (I.V)

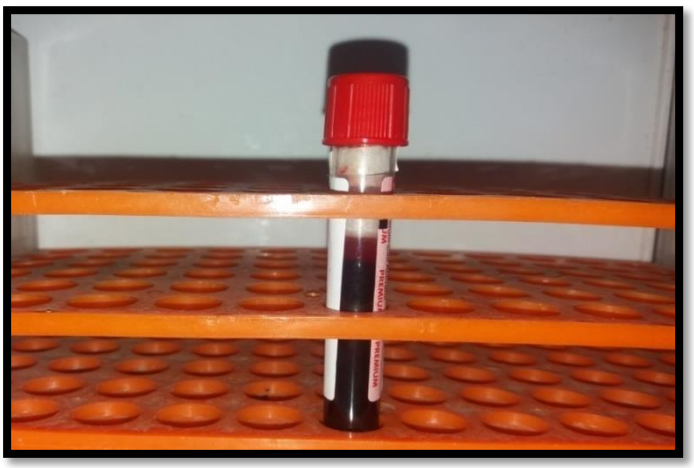


Figure 6: Collected blood sample

Figure 7 : Centrifuge machine

Figure 8: Placement of blood sample into centrifuge machine

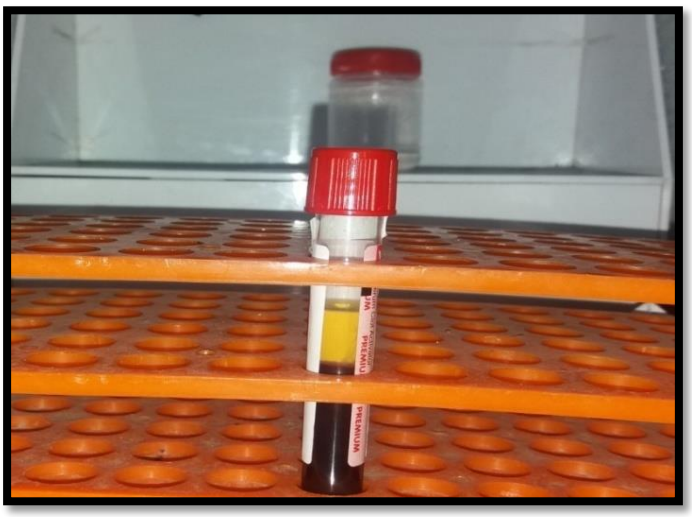


Figure 9: Centrifuged serum sample

Figure 10 : Albumin kit (Biosystems)

Figure 11 : Biochemical auto Analyzer for albumin estimation

RESULTS

This study was conducted to evaluate and compare the levels of serum albumin in patients with generalized chronic periodontitis, localized aggressive periodontitis and periodontally healthy individuals.

Totally 60 subjects were divided into three groups. Group- I consists of 20 healthy control subjects, Group-II consists of 20 Chronic generalised periodontitis patients, Group-III consists of 20 localized aggressive periodontitis, were selected from the outpatient section of Department of Periodontology, Adhiparasakthi dental college and hospital.

All clinical parameters were measured and blood samples were collected on the same day. Blood samples were sent for spectrophotometry analysis for albumin estimation. The obtained results were tabulated and the data were subject to statistical analysis.

STATISTICAL ANALYSIS

Obtained data was subjected to statistical analysis through SPSS (statistical package for social science).

The **unpaired t test** was used to assess the correlation between age, clinical parameters such as gingival bleeding index, probing depth, clinical attachment level and serum albumin level.

Table 2 : Mean age of all the three groups

Group I	**33.25**
Group II	**44.90**
Group III	**25.20**

Table 3 : Comparison of mean age between three groups

	Group I	Group II	Group III	T-value	P-value
Age	33.25±1.51	44.90±0.92		6.56	0.0000**
	33.25±1.51		25.20±1.30	4.02	0.0003**
		44.90±0.92	25.20±1.30	12.30	0.0000**

The mean age of group I was 33.25±1.51and group II was 44.90±0.92. On comparing the mean age between group I and group II the difference was found to be statistically significant. (p-value 0.0000)

The mean age of group I was 33.25±1.51 and group III was 25.20±1.30. On comparing the mean age between group I and group III the difference was found to be statistically significant. (p-value 0.0003)

The mean age of group II was 44.90±0.92 and group III was 25.20±1.30. On comparing the mean age between group II and group III the difference was found to be statistically significant (p-value 0.0000)

Chart 1: Mean age of all the three groups

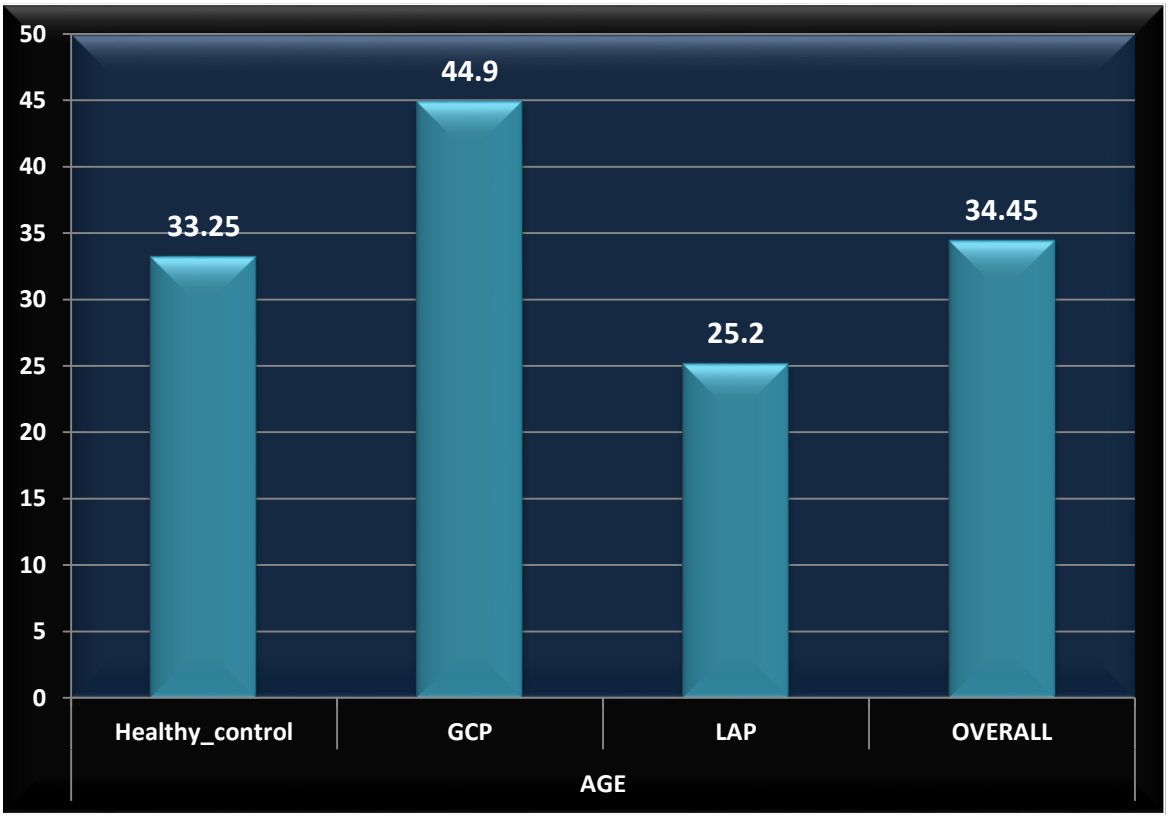


Table 4 : Mean GBI Score of all three groups

Group I	15.70 %
Group II	76.20 %
Group III	70.60%

Table 5: Comparison of mean GBI Score between three groups

	Group I	**Group II**	**Group III**	**T-value**	**P-value**
GBI	15.70±1.32%	76.20±2.61%		20.63	0.0000***
	15.70±1.32%		70.60±2.35%	20.28	0.0000***
		76.20±2.61%	70.60±2.35%	1.59	0.1201

The mean GBI score of group I was 15.70±1.32 % and group II was 76.20±2.61 %.On comparing GBI score between group I and group II the difference in the number of bleeding sites was found to be statistically significant (p-value 0.0000)

The mean GBI score of group I was 15.70±1.32 % and group III was 70.60±2.35 %. On comparing GBI score between group I and group III the difference in the number of bleeding sites was found to be statistically significant (p-value 0.0000)

The mean GBI score of group II was 76.20±2.61 % and group III was 70.60±2.35%. On comparing GBI score between group II and group III the difference in the number of bleeding sites was not found to be statistically significant. (p-value 0.1201)

Chart 2 : Mean GBI score of all the three groups

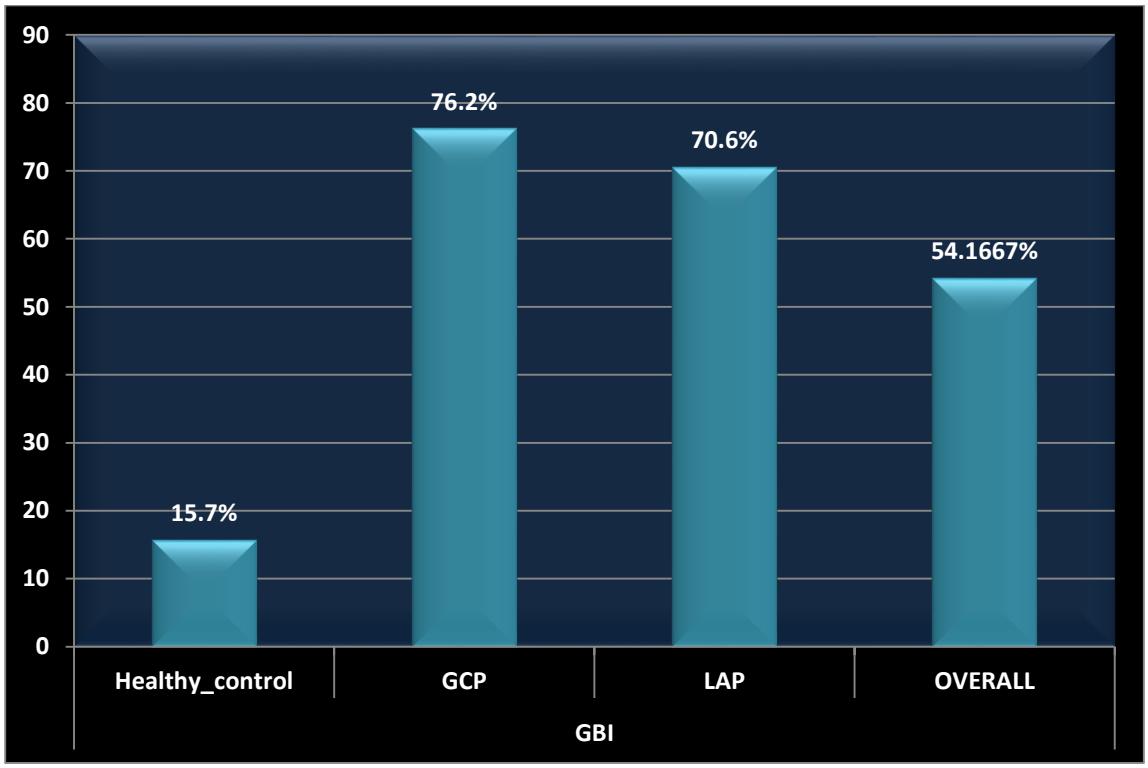


Table 6 : Mean probing depth of all three groups

Group I	1.22mm
Group II	3.98mm
Group III	3.66mm

Table 7 : Comparison of mean probing depth between three groups

	Group I	**Group II**	**Group III**	**T-value**	**P-value**
PD	1.22±0.04mm	3.98±0.13mm		19.49	0.0000***
	1.22±0.04mm		3.66±0.08mm	25.07	0.0000***
		3.98±0.13mm	3.66±0.08mm	1.97	0.0560

The mean probing depth of group I was1.22±0.04 mm and group II was 3.98±0.13mm. On comparing probing depth between group I and group II the difference in probing depth was found to be statistically significant.(p –value 0.0000)

The mean probing depth of group I was1.22±0.04mm and group III was 3.66±0.08 mm. On comparing probing depth between group I and group III the difference in probing depth was found to be statistically significant. (p –value 0.0000)

The mean probing depth of group II was 3.98±0.13mm and group III was 3.66±0.08mm. On comparing probing depth between group II and group III the difference in probing depth was not found to be statistically significant. (p –value 0.0560)

Chart 3 : Mean probing depth of all the three groups

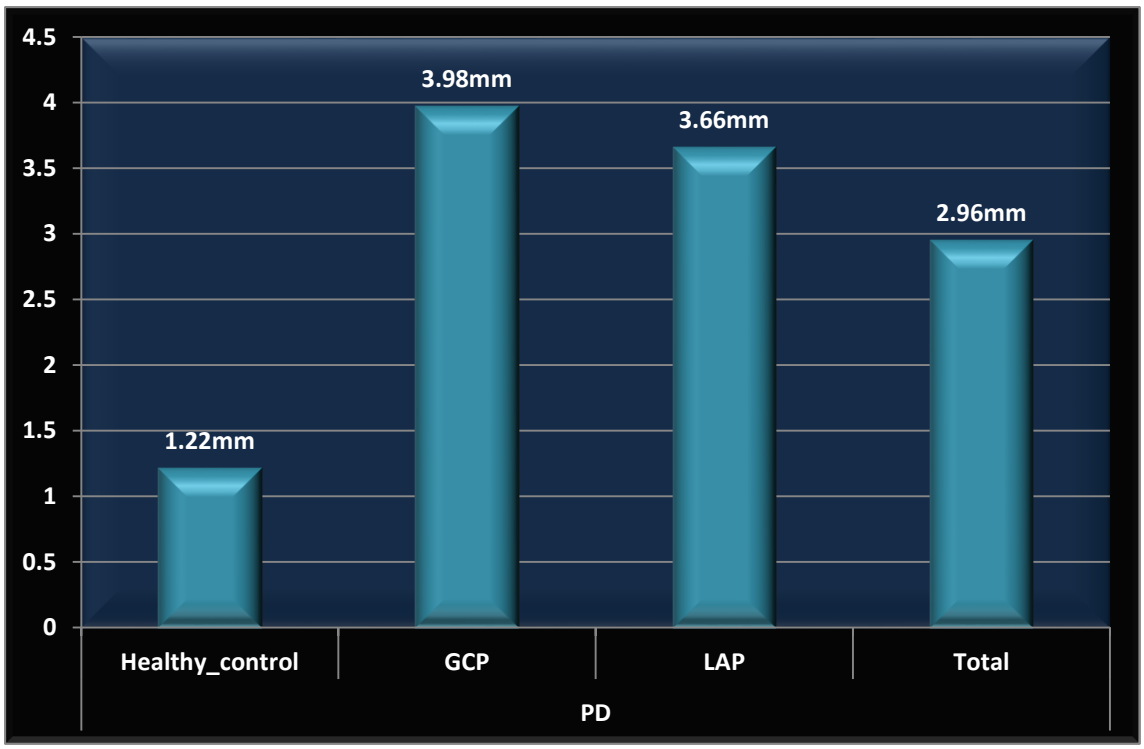


Table 8: Mean CAL of all three groups

Group I	1.34mm
Group II	4.40mm
Group III	4.17mm

Table 9: Comparison of mean CAL between three groups

	Group I	**Group II**	**Group III**	**T-value**	**P-value**
CAL	1.34±0.05mm	4.40±0.15mm		19.14	0.0000***
	1.34±0.05mm		4.17±0.07mm	30.95	0.0000***
		4.40±0.15mm	4.17±0.07mm	1.37	0.1774

The mean CAL of group I was 1.34±0.05mm and group II was 4.40±0.15mm. On comparing CAL between group I and group II the difference in CAL was found to be statistically significant.(p –value 0.0000)

The mean CAL of group I was 1.34±0.05mm and group III was 4.17±0.07mm. On comparing CAL between group I and group III the difference in CAL was found to be statistically significant. (p –value 0.0000)

The mean CAL of group II was 4.40±0.15mmand group III was 4.17±0.07mm. On comparing CAL between group II and group III the difference in CAL was not found to be statistically significant. (p – value 0.1774)

Chart 4 : Mean CAL of all the three groups

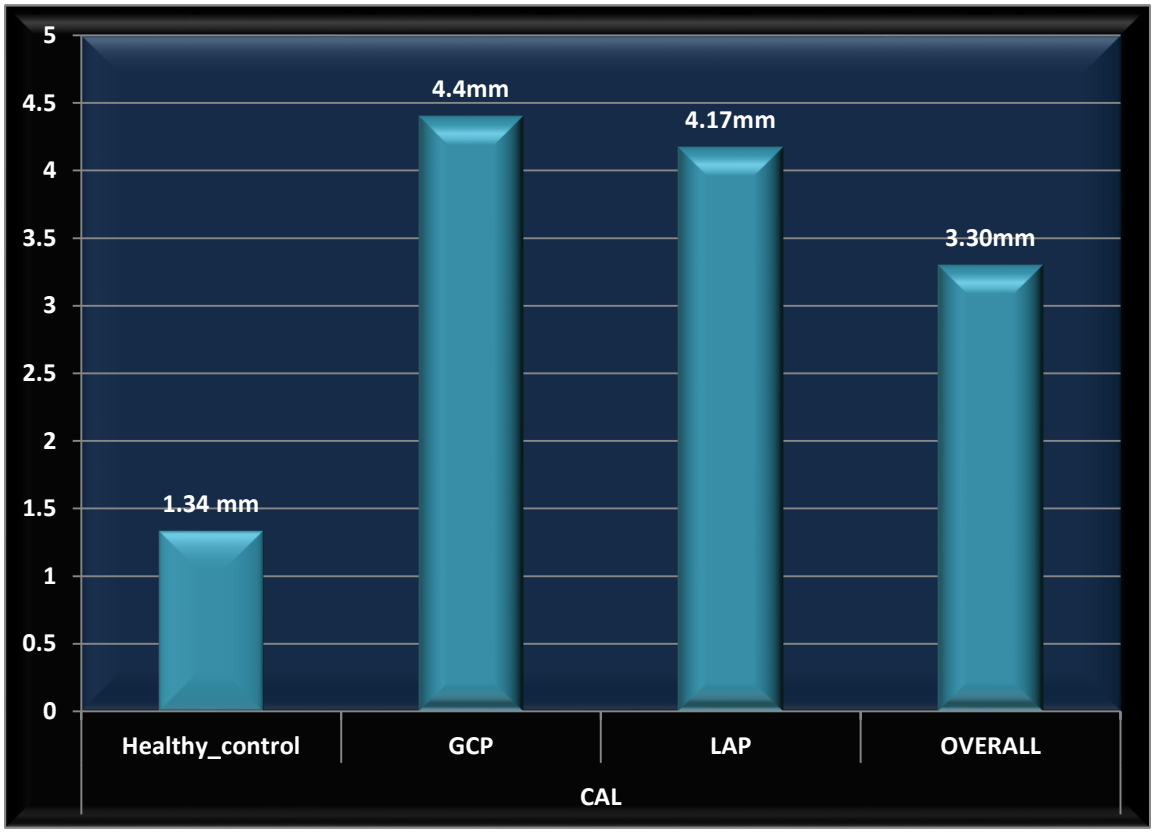


Table 10: Mean Serum Albumin level of all three groups

Group I	4.68 g/dl
Group II	4.12 g/dl
Group III	4.05g/dl

Table 11: Comparison of Mean Serum Albumin level between three groups

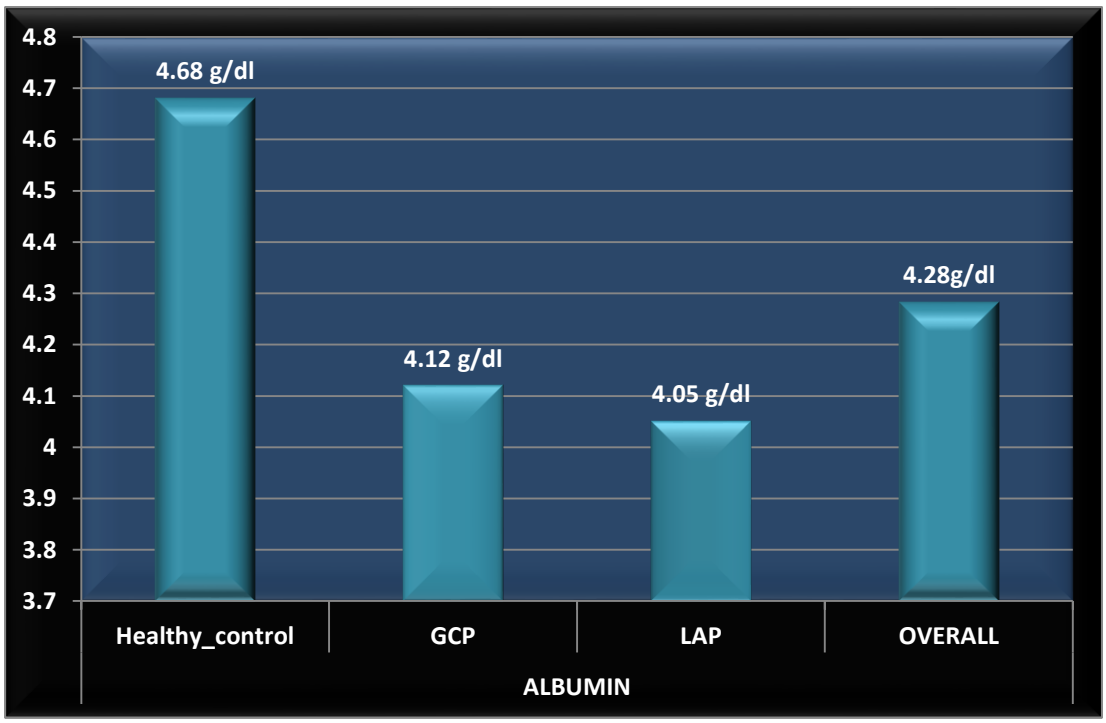
	Group I	**Group II**	**Group III**	**T-value**	**P-value**
	4.68±0.06g/dl	4.12±0.07g/dl		5.53	0.0000***
Albumin	4.68±0.06g/dl		4.05±0.06g/dl	6.72	0.0000***
		4.12±0.07g/dl	4.05±0.06g/dl	0.73	0.4722

The mean serum albumin level of group I was 4.68±0.06g/dl and group II was 4.12±0.07g/dl. On comparing serum albumin level between group I and group II the difference in serum albumin level was found to be statistically significant. (p –value 0.0000)

The mean serum albumin level of group I was 4.68±0.06 g/dl and group III was 4.05±0.06g/dl. On comparing serum albumin level between group I and group III the difference in serum albumin level was found to be statistically significant (p –value 0.0000)

The mean serum albumin level of group II was 4.12±0.07g/dl and group III was 4.05±0.06g/dl. On comparing serum albumin level between group II and group III the difference in serum albumin level was not found to be statistically significant (p –value 0.4722)

Chart 5 : Mean Serum Albumin level of all the three groups



DISCUSSION

This study was conducted at the Department of Periodontology, Adhiparasakthi Dental College and Hospital, Melmaruvathur (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. A total number of 60 subjects were enrolled in the study with the age range of 18 -50 years from the outpatient division of APDCH. 60 subjects were divided into three groups. Group-I consists of 20 healthy control subjects, Group-II consists of 20 chronic periodontitis patients, Group-III consists of 20 localized aggressive periodontitis

Serum albumin level evaluated was by collecting the blood samples from periodontally healthy individuals and patients with chronic generalised periodontitis and localized aggressive periodontitis.

Inflammation and malnutrition both reduce the albumin concentration by decreasing its rate of synthesis. Chronic diseases are associated with inflammation and the release of inflammatory cytokines such as IL-6, TNF α, which cause decrease in serum albumin. (**schalk et al 2004)**[58]

Periodontitis is defined as an inflammatory condition of the gingival tissues, characterized by loss of attachment of the periodontal ligament and the bony support of the tooth (**Genco et al 1990**).In periodontal diseases, bacteria trigger inflammatory host responses that cause destruction of the alveolar bone and periodontal connective

tissue. The individual characteristics that diminish the efficiency of host response may include medical factor such as malnutrition, which consistently impairs the innate and adaptive defences of the host, including phagocytic function, cell mediated immunity, complement, secretory antibody and cytokine production and function.

In the past few years various studies in human have been conducted and proved the relationship between serum albumin concentration and chronic periodontitis.

According to Hermann et al 1992 many conditions such as inflammatory status, liver and renal disease have been indicated to reduce serum albumin levels. Moreover malnutrition also may be monitored by means of serum albumin concentration **(Don kaysen 2004)[21].**

According to **Monjon et al 1999** in subjects who had teeth with periodontal pockets greater than 6mm, had significantly lower serum albumin concentration. **Tomoyo et al 2007** in his study revealed that patients with CAL >6mm had lower serum albumin concentration.

A longitudinal study by **Iwasaki M et al. (2008)** stated that there is a significant association between the number of periodontal disease events over 4 years and serum albumin levels. Serum albumin concentrations had a significant effect on periodontal disease progression among non smokers and serum albumin concentration is a significant risk predictor of periodontal disease progression among

elderly non smokers. **Shi D et al., (2008)** explored the characteristics of peripheral blood cellular and serum protein parameters in patients with Aggressive Periodontitis. Results showed albumin level and albumin globulin ratio were lower in Aggressive Periodontitis group than in the control group.

Ogawa et al., **2006** also found that, the mean CAL was a significant factor associated with lower albumin in community-dwelling elderly individuals.

Ibrahem HM *et al.*, (2006) Assessed the plasma antioxidant status in patients with periodontitis using serum albumin concentration as a criterion index and the results of the study showed that periodontitis patients had a significant decrease in the level of serum albumin than that of the healthy subjects ($p<0.05$), decreased level of serum albumin in patients with periodontitis, which indicates decreased antioxidant activity in patients with periodontitis on comparison with the healthy individuals.

A**mitha R *et al.*, *(2012)*** evaluated the association between serum albumin concentration and periodontitis. They found that, there is a inverse association between serum albumin concentration and loss of attachment in periodontitis patients and the reduction in serum albumin concentration in periodontitis group when compared to the healthy controls. S**andeep K *et al., 2014*** revealed the correlation between periodontal disease and serum albumin concentration in chronic periodontitis patients. Results showed there is significant decrease in

the level of serum albumin in patients with chronic periodontits when compared to periodontally healthy individuals.

Kaur N *et al., (2015)* Conducted a study to evaluate the relationship between the periodontal health status and serum albumin level. The results showed mean serum albumin levels for periodontally healthy individual (group I) was 4.815 g/dl and for chronic periodontitis (group II) the mean serum albumin levels was 4.219 g/dl and stated that the difference between serum albumin levels in group I and group II were found to be statistically significant ($p \leq 0.001$) and concluded that there is an inverse relationship between the serum albumin concentration and chronic periodontal disease.

All the studies conducted so far has determined the relationship between generalized chronic periodontitis and even comparison of the same with periodontally healthy individuals, but none of the study have compared the serum albumin level between patients having localized aggressive periodontitis with that of periodontally healthy individuals.

Hence the present study was conducted to compare and evaluate the serum albumin levels between periodontally healthy individual and patients with generalised chronic periodontitis and localized aggressive periodontitis. All clinical parameters including gingival bleeding index, probing depth and CAL, along with serum samples were collected.

The results indicated that, there was significant difference in the mean age between group I **33.25±1.51**and group II **44.90±0.92** with p – value **0.0000.** There was a significant difference in the mean age between group I **33.25±1.51**and group III **25.20±1.30** with p –value **0.0003** and also there is significant difference between group II **44.90±0.92** and group III **25.20±1.30** with p-value **0.0000** which indicates the younger individuals are affected by localized aggressive periodontitis.

The results indicated that, there was significant difference in the mean GBI score between group I **15.70±1.32** and group II **76.20±2.61** with p –value **0.0000**and also there was a significant difference in the mean GBI score between group I **15.70±1.32** and group III **70.60±2.35** with p –value 0.0000 but there is no significant difference between group II **76.20±2.61** and group III **70.60±2.35**with p-value **0.1201.** The percentage of bleeding sites is more in group II and group III which is a sign of active tissue destruction and presence of greater area of inflamed connective tissue that is cell rich, collagen poor tissue. Gingival bleeding on probing indicates an inflammatory lesion both in epithelium and in the connective tissue compared with healthy gingiva.

There is a significant difference in the mean probing depth between group I **1.22±0.04** and group II **3.98±0.13** with p –value **0.0000**.and also there was a significant difference in the mean probing depth between group I **1.22±0.04** and group III **3.66±0.08** with p –value

0.0000 but there is no significant difference between group II **3.98±0.13** and group III **3.66±0.08**with p –value **0.0560.**

There is a significant difference in the mean CAL between group I **1.34±0.05** and group II **4.40±0.15** with p –value**0.0000** and there was a significant difference in the mean CAL between group I **1.34±0.05** and group III**4.17±0.07** with p –value**0.0000** but there is no significant difference between group II **4.40±0.15** and group III **4.17±0.07**with p – value **0.1774**.

Increase in pocket depth and clinical attachment loss was found in group II and group III. This may be the result of the infection and interaction of selected bacterial species with that of the host immune response, which is characterized by the loss of connective tissue attachment that supports the dentition (cementum, periodontal ligament, and alveolar bone), and the formation of periodontal pockets that are colonized with Gram-negative, facultative, or anaerobic bacterial species. An intense inflammatory cell infiltrate will be recruited into the lesion that secretes pro-inflammatory mediators, including prostaglandin E2, IL-1, IL-6, and TNF. Moderate to severe periodontitis can precipitate an acute-phase response which will increase systemic markers of inflammation.

On comparison there is a significant difference in the mean serum albumin level between group I **4.68±0.06**and group II **4.12±0.07**with p –value0.0000 and there was a significant difference in the mean serum albumin level between group I **4.68±0.06** and group III

4.05±0.06with p –value0.0000, but there is no significant difference in serum albumin level between group II **4.12±0.07**and group III **4.05±0.06**with p –value **0.4722.**

Inflammation is a well known cause of hypoalbuminemia. During inflammation, cytokines such as TNF and IL-1 serve to shunt amino acids away from producing proteins that are nonessential to the inflammatory process toward positive acute phase proteins including globulins, fibrinogen and haptoglobin but negative acute phase proteins such as albumin, the synthetic rate drops during inflammation. The drop in concentration of albumin during inflammation can be significant, averaging 0.5g/dl in humans. Therefore serum albumin level found to be decreased during inflammation and release of inflammatory cytokine such as IL-1, IL-6 and TNF-α caused by periodontal disease. This may be the reason for reduction of serum albumin level in group II and group III patients.

Results of this study is in accordance to the study conducted by Iwasaki et al (2008), Shi D et al (2008), Ogawa et al (2006), Ibrahem HM *et al.*,(2006), Amitha R et al (2012), Kaur N et al (2015).

In the present study all clinical parameters shown to be increased significantly in patients with chronic generalized periodontitis (group II) and localized aggressive periodontitis (group III) when compared to periodontally healthy individual (group I).

All the clinical parameters in patients with chronic generalised periodontitis (group II) shown to be increased when compared to localized aggressive periodontitis (group III) but there is no statistically significant difference between these two groups.

There is a significant decrease in serum albumin level in patients with chronic generalized periodontitis (group II) and localized aggressive periodontitis (group III) when compared to periodontally healthy individual (group I)

There is decreased Serum albumin level in patients with localized aggressive periodontitis (group III) compared to patients with chronic generalised periodontitis (group II) but the difference was not statistically significant.

In this study we chose albumin as a periodontal disease marker because of its potential role in the periodontal disease progression of an individual. Serum albumin level found to be decreased during inflammation and release of various inflammatory cytokine such as IL-1, IL-6and TNF α which occurs in periodontal disease. Various clinical studies have proved the beneficial outcome of choosing serum albumin as periodontal disease risk marker and its good predictable value. The evaluation of serum albumin in laboratory is cheaper and the method of evaluation by spectrophotometry is made simplified by the advances in equipment to biochemical analyzer. Hence, Albumin can be used to rule out the periodontal disease activity in a short period of time. Another advantage of electing albumin as a biomarker of periodontal

disease progression is because of its low cost, which will be much useful for patients with poor socio-economic status.

The results of the present study signifies that there is a significant difference in all the clinical parameters which includes gingival bleeding index, probing depth and CAL in group-I when compared with group-II and group-III.

There is a statistically significant difference of age between all the three groups which includes group I, group II, group III. Lowest mean age group was found to be group III.

There is a significant decrease in serum albumin level in group II and group III compared to periodontally healthy individual (group I).

To our knowledge this is the first study to compare and evaluate the serum albumin level in patients with chronic generalised periodontitis, localized aggressive periodontitis with that of periodontally healthy individuals.

With the results of the present study, it could be suggested that serum albumin level could be used as a predictive marker to identify the severity of periodontitis. Further studies with large population size should be conducted to synergize the outcome of the present study.

CONCLUSION

The goal of periodontal diagnostic procedures is to provide useful information to the clinician on the periodontal disease type, location and severity; this information can serve as a basis for the planning of treatment and monitoring of disease.

Long established method of diagnosing periodontitis includes clinical parameter assessment and radiographic findings to evaluate periodontal tissue destruction. Although screening and diagnostic modalities for the early identification of periodontal disease is not significant along the traditional diagnostic method, a need for evolution of reliable diagnostic method to rule out periodontal disease in an early stage lead to the emergence of biomarkers in the of field of diagnosis.

To date, various inflammatory and immune mediators have been incriminated as biomarker in the periodontal tissue destruction. Serum albumin is a negative acute phase protein; Inflammation and malnutrition both reduce serum albumin concentration by decreasing its rate of synthesis. This suggests that periodontal disease severity might be indicated and monitored by the levels of serum albumin. Therefore, serum albumin can be used as a risk predictor for periodontal disease.

Evaluation of serum albumin by simplified method of spectrophotometry and its economical analysis cost, makes the serum

albumin as resilient and reliable one for the diagnosis of periodontal disease activity for any individual.

The results of the present study shows that, Serum albumin levels were decreased in patients with chronic generalised periodontitis and localized aggressive periodontitis when compared with that of periodontally healthy individuals.

The decreased level of albumin in serum were positively correlated with clinical parameters including gingival bleeding index, probing depth and CAL in patients with chronic generalised periodontitis and localized aggressive periodontitis.

With the limitations of the present study it could be concluded that there is inverse relationship of serum albumin level and periodontal diseases. So, serum albumin level can be used as a predictive marker to evaluate the periodontal disease activity. Further randomized clinical trials and longitudinal evaluations in a larger population would be required to support the observation of the present study.

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ADHIPARASAKTHI DENTAL COLLEGE AND HOSPITAL

DEPARTMENT OF PERIODONTICS

PROFORMA

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

GINGIVAL BLEEDING INDEX

7	6	5	4	3	2	1	1	2	3	4	5	6	7

PROBING DEPTH

palatal palatal palatal

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

Buccal labial buccal

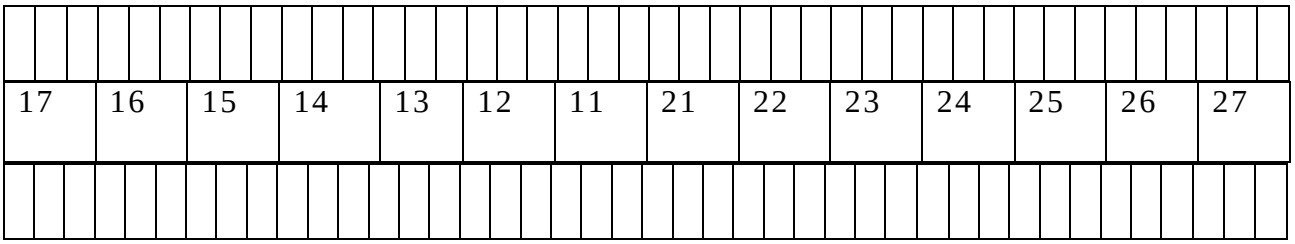
Lingual lingual lingual

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

Buccal labial buccal

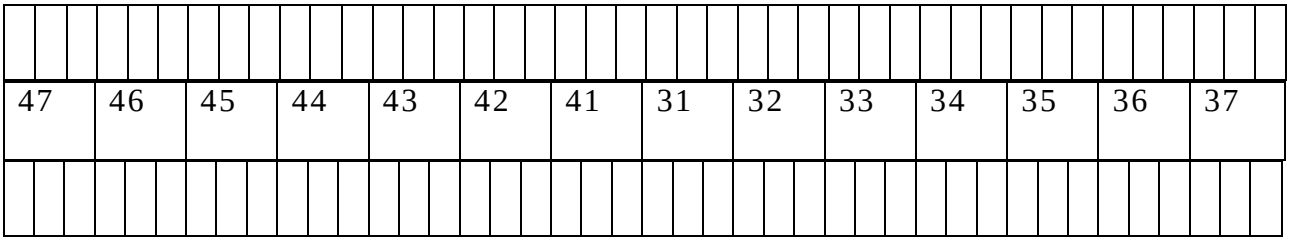
CAL

palatal palatal palatal



Buccal labial buccal

Lingual lingual lingual



Buccal labial buccal

DIAGNOSIS:

SERUM ALBUMIN LEVEL

PARTICIPANT INFORMED CONSENT FORM (PICF)

(English)

Protocol / Study number :_____

Participant identification number for this trial: _____

Title of project:_____ _____

_____

_____

Name of Principal Investigator: _____

Tel.No(s).____

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.

Date:(Signatures / Left Thumb Impression)

Place:

Name of the Participant: _____ _

Son / Daughter / Spouse of:_____ _

Complete postal address: _____

This is to certify that the above consent has been obtained in my presence.----------

Signatures of the Principal Investigator

Date:

Place:

பொது வாய்நோய் சிகிச்சைக்கான ஒப்புதல் படிவம்

துறை : _____

தேதி:

நோயாளியின் பெயர் :

வயது / பாலினம்:

புறநோயாளி எண்:

அறுவை சிகிச்சை மருத்துவ நிபுணரின் பெயர் :

சிகிச்சையின் பெயர்:_____

அளிக்கப்படும் மயக்க மருந்தின் வகை :

எனது தற்போதைய வாய் நலம்குறித்தும், அதற்கு உரிய சிகிச்சை முறைகளையும், மாற்று சிகிச்சை முறைகளையும் மற்றும் சிகிச்சை மேற்கொள்ளாவிடில் ஏற்படும் பின்விளைவுகளையும் பல் மருத்துவர் முழுமையாக என்னிடம் கூறினார். அதற்கான எனது சந்தேகங்களையும் பல் மருத்துவரிடம் 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.

நோயாளியின் உதவியாளர் :

பெற்றோரின் கையொப்பம் நோயாளியின் கையொப்பம்:

அறுவை சிகிச்சை நிபுணரின் கையொப்பம் :

மருத்துவரின் கையொப்பம்:

Omsakthi
ADHIPARASAKTHI
DENTAL COLLEGE & HOSPITAL
Recognised by Dental Council of India
Affiliated to The Tamilnadu Dr.M.G.R Medical University
A Unit of Adhiparasakthi Charitable, Medical, Educational & Cultural Trust

This Ethical Committee has undergone the research Protocol submitted by Dr.P.Indumathi, Post Graduate Student, Department of Periodontics, under the title "Estimation of Serum Albumin levels in Chronic Generalized Periodontitis and Localised Aggressive Periodontitis patients and Compare it with Periodontally Healthy Individuals" Ref no : 2016-MDS-BrII-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 recommendations 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.

Principal

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Urkund Analysis Result

Analysed Document:	new corrected main desertation 111.docx (D46310918)
Submitted:	12/29/2018 9:48:00 AM
Submitted By:	indukutty235@gmail.com
Significance:	10 %

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