

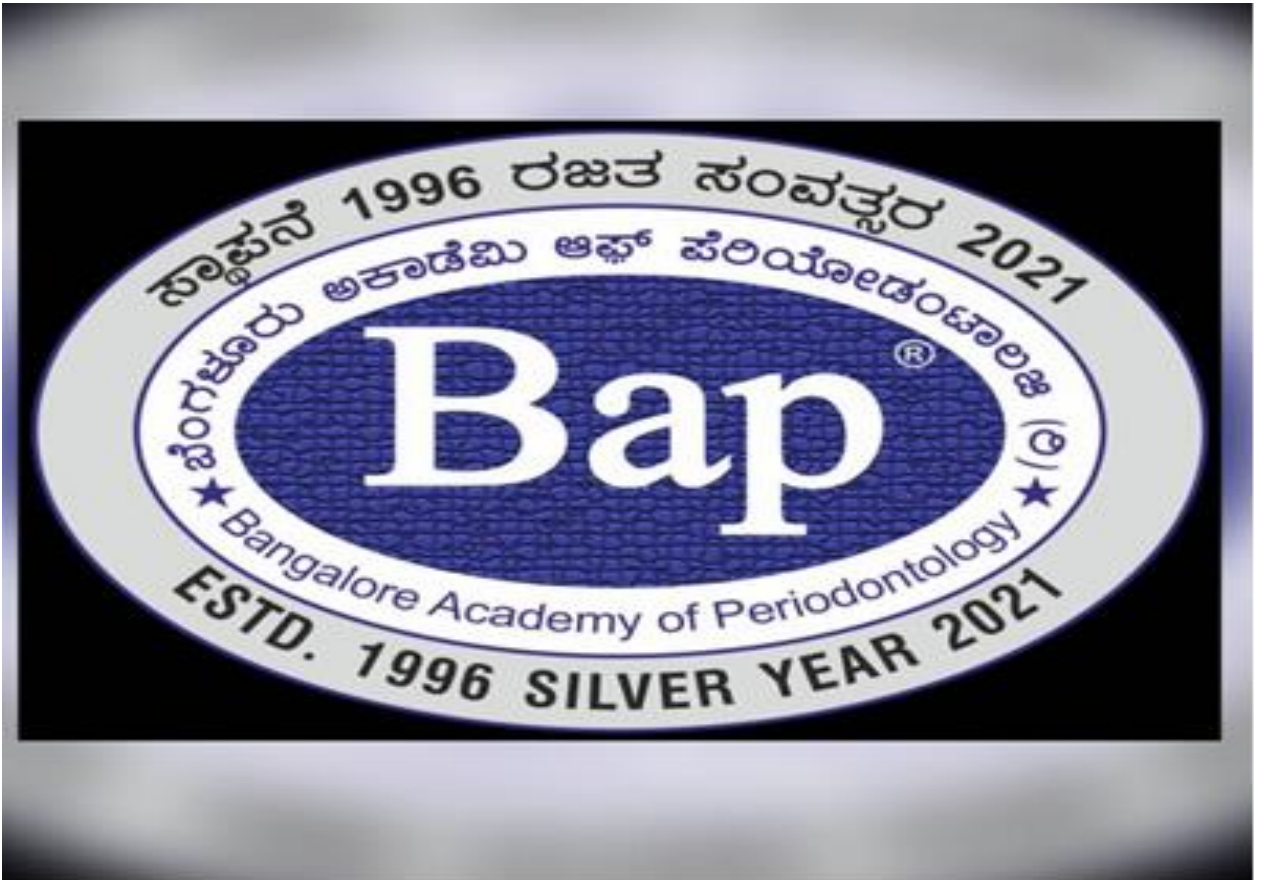
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# *Bangalore Academy of Periodontology*

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**Application for Research  
Projects for Grants  
for the Operation of Projects**



**1. Title of the Research Project:** “COMPARATIVE EVALUATION OF IMMUNOEXPRESSION OF A20 TUMOR NECROSIS FACTOR-ALPHA INDUCIBLE PROTEIN-3 (TNFAIP3) IN DIFFERENT PHENOTYPES OF PERIODONTITIS”. AN ANALYTICAL CROSS-SECTIONAL STUDY.

2.

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**3. Duration of Research Project:**

- a. Period which may be needed for collecting the data: 6 Months.
- b. Period that may be required for analyzing the data: 2 Months.

**4. Institution responsible for the research project:**

Name: Government dental college and Research Institute, Bengaluru.

Postal address: Fort, Victoria Hospital Campus, Bengaluru 560002

Telephone: 080-267050553,080-26703176.

E-mail: gdcricbangalore@gmail.com

Fax No:

**5. Institutional ethical clearance (IEC) and Project approval. Does the Project involve Human studies or Animal experiments?**

Ethical clearance has been obtained from the Institutional Ethical Committee and Review Board of Government Dental College and Research Institute, Bengaluru.

**No. GDCRI/IEC-ACM(2)/10/2020-21.**

Yes, the Patient's gingival tissues will be taken during periodontal surgery, tooth extraction, and crown lengthening procedures.

**6. The Head of the Department where the study is being done should ensure that there is no financial conflict of interest by the investigators.**

There is no conflict of interest between investigators and the Head of the Department.

# **“COMPARATIVE EVALUATION OF IMMUNOEXPRESSION OF A20 TUMOR NECROSIS FACTOR-ALPHA INDUCIBLE PROTEIN-3 (TNFAIP3) IN DIFFERENT PHENOTYPES OF PERIODONTITIS”. AN ANALYTICAL CROSS-SECTIONAL STUDY.**

**Summary:** Periodontitis is a multifactorial disease in which the host's response to the bacteria also contributes to the progression of the disease. A20 (Tumor necrosis factor  $\alpha$ -induced protein 3 or TNFAIP3) is a ubiquitin-editing enzyme that mainly functions as an endogenous inflammation regulator through nuclear factor termination (NF)- $\kappa$ B activation as part of a negative feedback loop. Hence this prospective cross-sectional study evaluates the levels of A20 in different phenotypes of periodontitis. The present study might give an insight into the host response of different phenotypes of periodontitis.

**Keywords:** TNFAIP-3, NF- $\kappa$ B pathway, Periodontitis, Immunoexpression, ubiquitin editing enzyme, ELISA.

## **1. INTRODUCTION:**

Periodontitis is the most common oral disease and is a chronic inflammatory disease initiated by the microorganisms present in the dental biofilm, leading to the destruction of tooth-supporting tissues. It is a consequence of deregulated immune response initiated by the dysbiotic oral microflora.

A20 (Tumor necrosis factor- $\alpha$ -induced protein 3) is a pleiotropically expressed ubiquitin editing enzyme capable of interfering with Nuclear factor kappa light chain enhancer of activated B-cells (NF- $\kappa$ B), interferon regulatory factor (IRF), and c-jun N-terminal (JNK) signaling cascades, all of which are being implicated with periodontal disease pathogenesis. A20 serves as a critical modulator of inflammation and cell death in numerous tissues and diseases due to the cooperative activity of its deubiquitinating and ubiquitin ligase domains. A study showed that A20 attenuates the inflammatory response to *Porphyromonas gingivalis*, a keystone periodontal bacterium, by interfering with NF-  $\kappa$ B signaling in macrophages.<sup>1</sup>

Previous studies revealed the immunoexpression levels of A20 (TNFAIP3) in gingival epithelial cells about periodontal disease.<sup>2</sup>

The Consensus report of the current classification concluded that current evidence does not support the distinction between chronic and aggressive periodontitis,<sup>3</sup> as defined by the 1999 Classification Workshop, as two separate diseases; however, periodontal literature is replete with articles giving evidence of aggressive periodontitis as a separate disease entity.

Hence, the present study is being conducted to explore any difference in the immunological profile between the grade A B and grade C periodontitis as assessed by immune expression of A20, a regulator of the NF- $\kappa$ B pathway.

## **2. REVIEW OF LITERATURE: -**

A study conducted by Yaji Li demonstrated that A20 acts as a regulator of inflammatory response in gingival keratinocytes through its effect on NF- $\kappa$ B signaling and desensitizes cells to Bacteria and cytokine-induced apoptosis in the oral mucosa.<sup>1</sup>

A study conducted by Hong J-Y et, al. to know the anti-inflammatory and anti-osteoclastogenic effects of zinc finger protein A20 overexpression in periodontal ligament cells revealed that A20 was upregulated in the gingival tissues and nicotine-stimulated periodontal ligament cells, indicating the A20 as a downstream signaling molecule in periodontal inflammation.<sup>2</sup>

According to the new periodontitis classification scheme, a disease that is previously recognized as “chronic” or “aggressive” are now grouped under a single category (“periodontitis”) and is further characterized based on a multi-dimensional staging and grading system. Staging is dependent upon the severity of disease at presentation and the complexity of disease management, while grading provides information about biological features of the disease including the rate of disease progression.<sup>3</sup>

A study was done by Katie.E. Crump et.al, to understand the interplay of toll-like receptors 9, myeloid cells, and deubiquitinase A20 in periodontal inflammation. It concluded that A20 was modestly up-regulated in human gingival tissue specimens from chronic Periodontitis. Toll-like receptor-9 (TLR-9) modulates periodontal disease progression at both the cellular & molecular level & identifies A20 as a novel downstream signaling molecule in the course of periodontal inflammation.<sup>4</sup>

A study was conducted to evaluate the anti-inflammatory response of A20 (TNFAIP3) in the oral mucosa by restraining NF- $\kappa$ B Activity. It concluded that depletion of A20 using gene editing in human macrophage-like cells (THP-1) significantly increased cytokine secretion,

whereas overexpression of A20 using Lentivirus infection dampened the cytokine production following bacterial challenge through modulating NF- $\kappa$ B activity. These showed the physiological role of A20 as a negative regulator of inflammation.<sup>5</sup>

This study present compares the immunoexpression of A20 (TNFAIP-3) in different grades of Periodontitis using ELISA.

### **3. OBJECTIVES OF THE STUDY:**

1. To estimate and compare the immunoexpression levels of A20 (TNFAIP3) in gingival tissues in periodontally healthy and periodontitis subjects. (Grade A Grade B and Grade C).
2. To find out the relationship between A20 (TNFAIP3) immunoexpression in periodontal health and individuals with periodontitis (Grade A Grade B and Grade C) with the clinical parameters periodontal.

### **4. METHODOLOGY:**

#### **SOURCES OF THE DATA**

Subjects undergoing periodontal surgery or surgical crown lengthening will be screened and selected. It will be made clear to the potential subjects that participation will be voluntary and written informed consent will be obtained from those who agree to participate.

#### **GENERAL INCLUSION AND EXCLUSION CRITERIA:**

##### **A) INCLUSION CRITERIA:**

1. Subjects in good general health.
2. Age group of 19-60 years.
3. Both female and male.

##### **B) EXCLUSION CRITERIA:**

- 1) Pregnant and lactating women.
- 2) Any other systemic disease that can alter the periodontal disease course.

- 3) Subjects on current antibiotics and anti-inflammatory drugs that may alter the course of periodontal disease.
- 4) Subjects who have received periodontal therapy in the past 3 months.
- 5) Subjects who are smokers.
- 6). Necrotizing periodontitis.
- 7). Periodontitis as a direct manifestation of systemic disease.
- 8). Periodontal abscess.
- 9). No endodontic therapy at the site of sample collection.

### **SAMPLE SIZE CALCULATION:**

The Present study is to the comparative evaluation of A20 in different stages of periodontitis among three different groups. Considering the effect size of 0.35 (medium; the ratio of mean difference and pooled Standard deviation), the power of the study (1- $\beta$ ) =80%, type 1 error 5%, the sample size was calculated using G\*power software ver. 3.1.9.2.

**F tests - ANOVA:** Fixed effects, omnibus, one-way

**Analysis:** A priori: Compute the required sample size

<b>Input:</b>	Effect size f	=	0.35
	$\alpha$ err prob	=	0.05
	Power (1- $\beta$ err prob)	=	0.80
	Number of groups	=	3

<b>Output:</b>	Noncentrality parameter $\lambda$	=	10.2900000
	Critical F	=	3.1093105
	Numerator df	=	2
	Denominator df	=	71
	Total sample size	=	75
	Actual power	=	0.74410

**The total calculated sample size is 75**

**STUDY DESIGN:** This study is a cross-sectional study with 3 balanced arms to evaluate the A20 immunoexpression in normal healthy periodontium and Periodontitis.

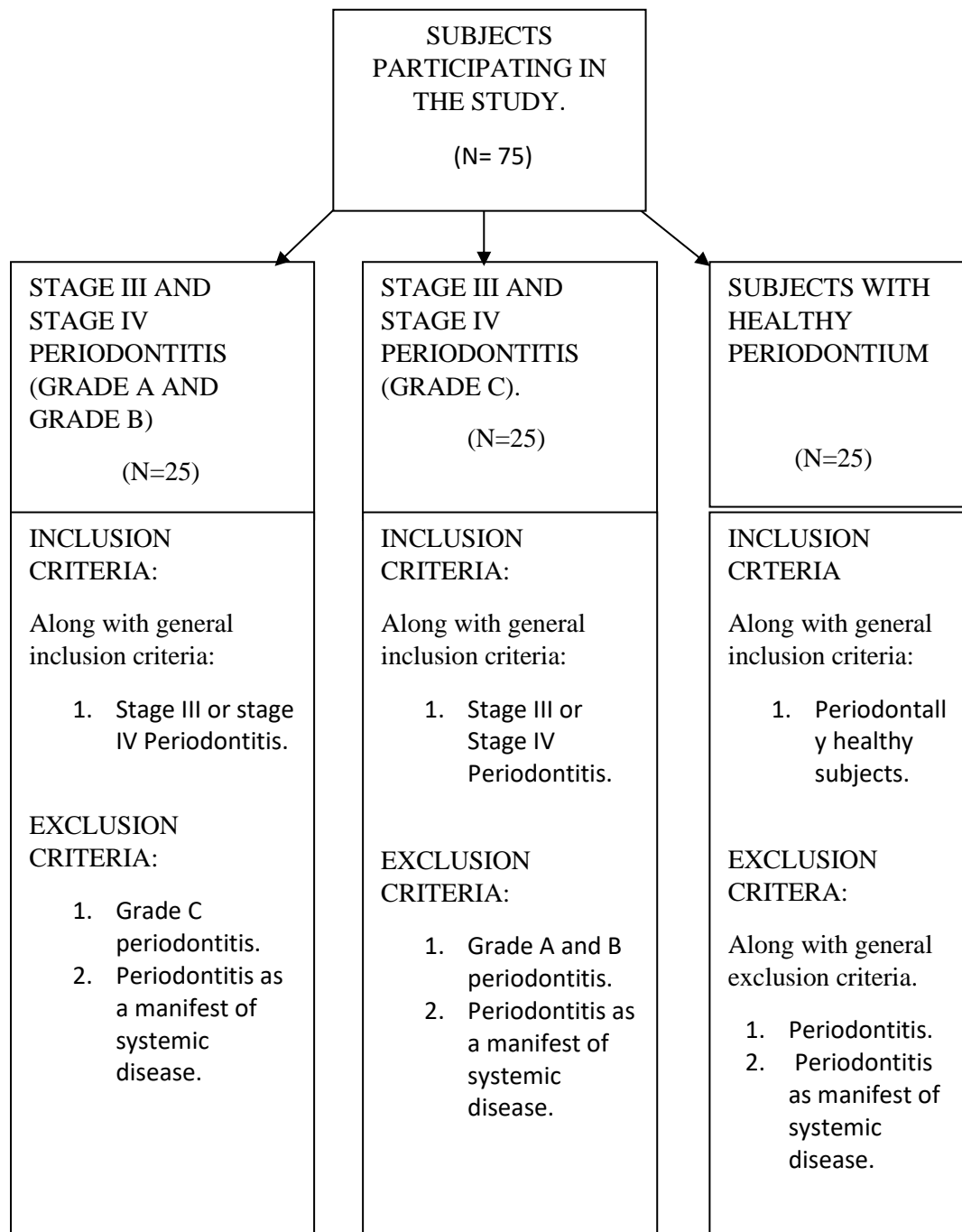
**Group 1-** Patients with stage III and IV (Grade A and Grade B) slow and a moderate rate of progressive periodontitis with Interdental clinical attachment loss of CAL  $\geq 5$  mm at the site of greatest loss, probing depth of  $\geq 6$ mm, vertical bone loss  $\geq 3$ mm tooth loss due to periodontitis  $\geq 4$  Class II and class III furcation involvement, .<sup>3</sup>

**Group 2-** Patients with stage III and IV (Grade C) rapid rate of progressive Periodontitis with Interdental clinical attachment loss of CAL  $\geq 5$  mm at the site of greatest loss, probing depth of  $\geq 6$ mm, vertical bone loss  $\geq 3$ mm tooth loss due to periodontitis  $\geq 4$  class II and class III furcation involvement .<sup>3</sup>

**Group 3** – Subjects with healthy periodontium undergoing extraction for orthodontic purposes or undergoing surgical Crown Lengthening procedures.

Healthy periodontium is defined as the absence of bleeding on probing, erythema and edema, patient symptoms and attachment, and bone loss at the site. (According to the Consensus report of workgroup 1 of the 2017 World Workshop on the Classification of Periodontal And Peri-Implant Diseases And Conditions.)<sup>3</sup>





## **METHODS OF COLLECTION OF DATA:**

Gingival tissue samples around the tooth will be obtained from the selected volunteers immediately after tooth extraction or during surgical crown lengthening procedure under local anesthesia in healthy subjects and periodontal surgery in periodontitis subjects. Each subject will contribute one sample. The incision of the tissues will be given at 3 mm sub-marginal and include the entire marginal gingiva of

the labial surface of the tooth being extracted in case of periodontal surgery. Therefore, the specimen will consist of the gingival margin, the sulcular epithelium, and the gingival connective tissues. The patient's Gingival biopsies will be obtained at different times from different subjects and stored under  $-70^{\circ}\text{C}$ . On the day of the assay, the biopsies will be allowed to thaw and 20-50 mg of wet tissue will be homogenized with the aid of a homogenizer. The Homogenates will be centrifuged to remove debris and insoluble material & will be assayed by Enzyme-Linked Immune-Sorbent Assay(ELISA). The ELISA will be carried out according to the manufacturer's guidelines.

## **5. STATISTICAL ANALYSIS:**

The data collected were entered into an excel spreadsheet. Data will be analyzed using the Statistical Package for Social Sciences (SPSS) version 24 (SPSS Inc., Chicago, IL, USA). The descriptive and inferential analysis will be done. Discrete data will be represented with frequency & proportion and continuous data will be represented by mean (SD). The normalcy of data will be determined using Kolmogorov-Smirnov Test and the parametric/non-parametric test will be applied accordingly based on the type and pattern of data. Chi-Square Test was used to find out the difference between proportions. The mean difference among the group variables will be determined by the One Way ANOVA/Kruskal Wallis test. Any additional analysis will be done based on the data pattern. Statistical significance will be considered at  $p < 0.05$  (confidence interval of 95%).

## **6. EXPECTED OUTCOMES:**

The challenge in studying rapid and chronic inflammatory conditions by characterizing the complex network of biological and molecular pathways, how they are regulated, and their ability to communicate with one another and facilitate the proinflammatory, anti-inflammatory, and resolution phases of the disease reveals the immune response in the Aggressive Periodontitis, chronic periodontitis, and healthy individuals.

## **7. LIMITATIONS OF THIS STUDY:**

The present study evaluates only one factor in the phenotypes of periodontitis, other factors are not being considered.

## **8. FUTURE PLANS BASED ON EXPECTED OUTCOMES:**

This study involves the New advances in the understanding of downstream regulation of inflammatory signaling and its links to the restoration of homeostasis offer promise in translational clinical research. There has been growing interest in exploiting components of ubiquitination machinery as therapeutic targets and this also offers great potential for targeted therapies.

## **9. TIMELINES:**

Details of activities to be carried out along with timelines during

Preparatory phase: - June 2022 -July 2022.

Data collection: - July 2022 – September 2022.

Analysis: - October 2022.

Report writing: - November 2022.

## **10. INSTITUTIONAL SUPPORT:**

ELISA reader and processing will be done in the Department of Periodontology, GDCRI, Bengaluru.

## **11. LIST OF REFERENCES:**

1. Li Y, Mooney EC, Xia XJ, Gupta N, Sahingur SE. A20 Restricts Inflammatory Response and Desensitizes Gingival Keratinocytes to Apoptosis. *Front Immunol.*2020;11:365.
2. Hong JY, Bae WJ, Yi JK, Kim GT, Kim EC. Anti-inflammatory and anti-osteoclastogenic effects of zinc finger protein A20 overexpression in human periodontal ligament cells. *J Periodontal Res.* 2016;51(4):529-39.
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5. Yajie Li, Erin C. Mooney, Sara E. Holden, Xia-Juan Xia, A20 orchestrates inflammatory response in the oral Mucosa through Restraining NF-kB Activity. *J Immunol* 2019;202:2044-2056: