APPLICATION FOR GRANT-IN-AID OF AD-HOC RESEARCH PROJECT

Section A GENERAL

1. Title of the Research Project:

COMPARATIVE EVALUATION OF IMMUNOEXPRESSION OF IL-17 A AND p53 IN GINGIVAL TISSUES OF PATIENTS ON AMLODIPINE WITH OR WITHOUT DRUG INDUCED GINGIVAL ENLARGEMENT

– AN ANALYTICAL CROSS-SECTIONAL STUDY

2. Name and Designation of

a. Candidate & Email: **DR. ARYA RAVINDRANATH**
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DEPARTMENT OF PERIODONTOLOGY
GOVERNMENT DENTAL COLLEGE AND RESEARCH INSTITUTE, BENGALURU

Investigator- 1 & Email: **DR. PURNIMA BANDARI**
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Investigator- 2 & Email: **Dr. SMITHA K**
PROFESSOR AND HEAD
DEPARTMENT OF PERIODONTOLOGY,
GOVERNMENT DENTAL COLLEGE AND RESEARCH INSTITUTE, BENGALURU.

3. Duration of Research Project: 13 months (April 2022- April 2023)

a. Period which may be needed for collecting the data: 10 months (April 2022- January 2023)

b. Period that may be required for analysing the data: 3 months (February 2023- April 2023)

4. Institution responsible for the research project:

Name: **GOVERNMENT DENTAL COLLEGE AND RESEARCH INSTITUTE, BENGALURU**

Postal address: Government Dental College & Research Institute, Bangalore, Victoria Hospital Campus, Fort, Bangalore, 560002

Telephone: 080-26705053 / 26703176

E - mail: gdcribangalore@gmail.com

Fax No: 080-26705053 / 26703176

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

yes

7. Is radio tagged material proposed to be used in the project either for clinical trials or experimental purposes? If so, clearance from Nuclear Medicine Committee, Bhabha Atomic Research Centre, Mumbai, indicating should be attached.

no

8. Does the Project involve recombinant DNA/ Genetic engineering work? If so, Project should be examined and certificate by the Institutional Bio safety Committee (IBSC) to be enclosed. Guidelines for constitution of IBSC can be obtained from Secretary, Department of Biotechnology, CGO Complex, Lodhi Road, New Delhi-110003

no

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

yes

DECLARATION AND ATTESTATION

i. I/We have read the terms and conditions for Research Grant. All necessary Institutional facilities will be provided if the research project is approved for financial assistance.

ii. I/We agree to submit within one month from the date of termination of the project the final report and a list of articles, both expendable and non-expendable, left on the closure of the project.

iii. I/We agree to submit utilization certificate with statement of accounts

iv. I/We agree to submit (online) all the raw data (along with descriptions) generated from the project to the BAP Data Repository within one month from the date of completion /termination of the project.

Signature of the:

a. Candidate Dr. Arya Ravindranath Arya

b. Investigator- 1 Dr. Purnima Bandari

c. Investigator- 2 Dr. Smitha K.

d. Name and signature of Guide Dr. Purnima Bandari

e. Head of the Department Dr. Smitha K.

f. Signature of the Head of the Institution with seal

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Dean Cum Director,
Govt. Dental College &
Research Institute
Bangalore

Date: 16/05/2022

Section B

1. TITLE OF THE PROPOSED RESEARCH PLAN:

COMPARATIVE EVALUATION OF IMMUNOEXPRESSION OF IL-17 A AND p53 IN GINGIVAL TISSUES OF PATIENTS ON AMLODIPINE WITH OR WITHOUT DRUG INDUCED GINGIVAL ENLARGEMENT – AN ANALYTICAL CROSS-SECTIONAL STUDY

2. Summary:

Background:

Gingival enlargement is a serious adverse effect that accompanies with the use of amlodipine, a calcium channel blocker derivative which adversely affects oral hygiene and impairs the oral function of medicated individuals.

Novelty:

Considering apoptosis mediators may play an important role in the pathogenesis of drug-induced gingival enlargement. The study is conducted to elucidate the combined role of IL-17A and p53 levels in the pathogenesis of amlodipine induced gingival enlargement.

Objectives:

To estimate and compare the immune expression levels of IL 17A and p53 as assessed by Enzyme-Linked Immunosorbent Assay (ELISA) in periodontally healthy subjects, patients on amlodipine with and without gingival enlargement and plaque induced inflammatory gingival enlargement.

Methods: The subjects enrolled will be categorized into four groups: -

GROUP I: - subjects under amlodipine medication who have gingival enlargement will be included in the study as the amlodipine responders. (n=12)

GROUP II: - subjects under amlodipine medication but no gingival enlargement. (non-responders) (n=12)

GROUP III: - Systemically healthy subjects with plaque induced chronic inflammatory gingival enlargement. (n=12)

GROUP IV: - Periodontal healthy subjects. (n=12)

Gingival tissue samples will be obtained from the selected volunteer during tooth extraction / surgical crown lengthening procedure /gingivectomy. The tissue samples will be thawed and homogenized with the aid of a homogenizer. The Homogenates will be assayed by Enzyme Linked Immunosorbent Assay (ELISA).

Expected outcome:

The pathogenesis of amlodipine induced gingival enlargement is multifactorial. Studies have shown that inflammation plays the most important role in the induction of apoptosis and proliferation in gingival tissues. The present study is expected to show that there is combined role of IL 17a and p53 in the pathogenesis of amlodipine induced gingival enlargement due to its interaction with amlodipine and its role in apoptosis and inflammation.

3. KEYWORD:

Gingival enlargement, IL 17A, p53, apoptosis mediators, amlodipine

4. ABBREVIATIONS:

CsA - cyclosporin A

EMT -epithelial-mesenchymal transition

ELISA - Enzyme linked immunosorbent assay

GCF – gingival crevicular fluid

OPG - orthopantomogram

5. **BACKGROUND:**

Amlodipine, a calcium channel blocker derivative, is frequently used by patients with high blood pressure, angina and myocardial infarction. Gingival enlargement is a serious adverse effect that accompanies with the use of amlodipine, which adversely affects oral hygiene and impairs the oral function of medicated individuals. It is important to elucidate the underlying mechanisms involved in drug-induced gingival enlargement, since the replacement of drugs is not always possible.

Apoptosis plays an important role in the maintenance of tissue homeostasis. Considering that apoptosis mediators may play a role in the pathogenesis of drug-induced gingival enlargement. The tight regulation of apoptosis is modulated by various transmembrane proteins such as p53.

Interleukin-17A (IL-17A) is known as a proinflammatory cytokine, but current

studies indicate that it has a role in fibrotic disorders and epithelial-mesenchymal

transition (EMT).[1]

p53 protein is encoded by p53 tumor suppressor gene (p53 gene) which is located on short arm of chromosome 17. The p53 induces apoptosis of transformed cell with severe DNA damage and therefore is referred to as “guardian of genome”.[2]

To our knowledge, no study has elucidated the combined role of IL-17A and p53 levels in the pathogenesis of amlodipine induced gingival enlargement. This study is undertaken to evaluate the immune expression of p53 and IL-17A in gingival tissues of patients on amlodipine with or without drug induced gingival enlargement.

6. LITERATURE REVIEW: -

A study was conducted on 29 Turkish subjects to figure out the possible role of IL-17A in amlodipine-induced gingival enlargement which was assessed by immunohistochemistry and Enzyme linked immunosorbent assay (ELISA) kits. In this study, elevated IL-17A expression regardless of inflammation shows that amlodipine might cause an increase of IL-17A in gingival tissues. This increase might induce fibrotic changes and EMT in gingival enlargement tissues.[1]

A study was conducted where 20 renal transplant patients exhibiting cyclosporin A (CsA) induced gingival overgrowth and 15 systemically healthy gingivitis patients were included and GCF samples were obtained to analyse the levels of p53, bcl2 and IL15 by ELISA. The finding suggested that IL-15 may play a role in the pathogenesis of CsA-induced gingival enlargement due to its interactions with CsA and its role in apoptosis and inflammation. p53 and bcl-2 levels were below the minimum detectable level in all GCF samples analyzed.[2]

An in vivo study was conducted in mice to examine the role of IL-17 signaling and related cytokines in hepatic fibrosis induced by either bile duct ligation (BDL) or carbon tetrachloride (CCl4). The results showed that in response to liver injury, levels of IL-17A and its receptor increased. IL-17A increased receptor appeared to promote fibrosis by facilitated production of IL-6, IL-1, and tumor necrosis factor-alpha by inflammatory cells and increased expression of transforming growth factor-1, a fibrogenic cytokine.[3]

A study was done in Cyclosporin A-induced gingival hyperplasia mouse model to examine the expression of cytokines including IL-17, IL-6 and growth factors (EGF and TGF-b) in gingival tissue was determined by real time RT-PCR. The results suggested that IL-6 and EGF play important roles in the pathogenesis of gingival hyperplasia, but IL-17 and TGF-b do not.[4]

7. NOVELTY/ INNOVATION: -

The pathogenesis of gingival enlargement is not fully elucidated. Considering apoptosis mediators may play an important role in the pathogenesis of drug-induced gingival enlargement. To our knowledge, no study has elucidated the combined role of IL-17A and p53 levels in the pathogenesis of amlodipine induced gingival enlargement. This study is undertaken to evaluate the immune expression of p53 and IL-17A in gingival tissues of patients on amlodipine with or without drug induced gingival enlargement.

8. **OBJECTIVES OF THE STUDY: -**

To estimate and compare the immune expression levels of IL 17A and p53 as assessed by Enzyme-Linked Immunosorbent Assay (ELISA) in periodontally healthy subjects, patients on amlodipine with and without gingival enlargement and plaque induced inflammatory gingival enlargement.

9. <u>METHODOLOGY:</u>

i. <u>STUDY DESIGN: -</u>

The is an **analytical cross-sectional study.**

After obtaining approval and clearance from the institutional ethical committee the subjects fulfilling the inclusion and exclusion criteria will be enrolled for the study. The subjects enrolled will be categorized into four groups: -

GROUP I: - subjects under amlodipine medication who have gingival enlargement

will be included in the study as the amlodipine

responders. (n=12)

GROUP II: - subjects under amlodipine medication but no gingival enlargement. (non-responders) (n=12)

GROUP III: - Systemically healthy subjects with plaque induced chronic inflammatory gingival enlargement. (n=12)

GROUP IV: - Periodontal healthy subjects. (n=12)

(Healthy periodontium is defined as absence of bleeding on probing, erythema and oedema, patient symptoms and attachment and bone loss at the site without any gingival enlargement.)

Informed Written consent will be taken from all the participating subjects in patient's preferred language/Audio visual recording.

SOURCES OF THE DATA: -

A purposive sample which includes 48 subjects will be selected from the outpatient section, Department of Periodontology, Government Dental College and Research Institute, Bengaluru who agree to participate in the study based on the inclusion and exclusion criteria. Participation in the study is voluntary and written informed consent will be obtained from those who agree to participate.

STUDY PERIOD: - 18 months (March 2021- September 2022).

PLACE OF THE STUDY: -

Department of Periodontology,
Government Dental College and Research Institute, Bengaluru.

ii. SAMPLE SIZE CALCULATION: -

The Present study is to find the immune expression of IL-17A and p53 among the four different Groups. Considering the effect size of 0.51 (mean difference and pooled Standard deviation), power of the study (1-β) =80%, type 1 error 5%, 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 required sample size

Input: Effect size (f) = 0.51

α err prob = 0.05

Power (1-β err prob) = 0.80

Number of groups = 4

Output: Noncentrality parameter λ = 12.4848000

Critical F = 2.8164658

Numerator df = 3

Denominator df = 44

Total sample size = 48

Actual power = 0.8198753

The calculated sample size is 48, 12 in each group.

GENERAL INCLUSION AND EXCLUSION CRITERIA: -

A. INCLUSION CRITERIA: -

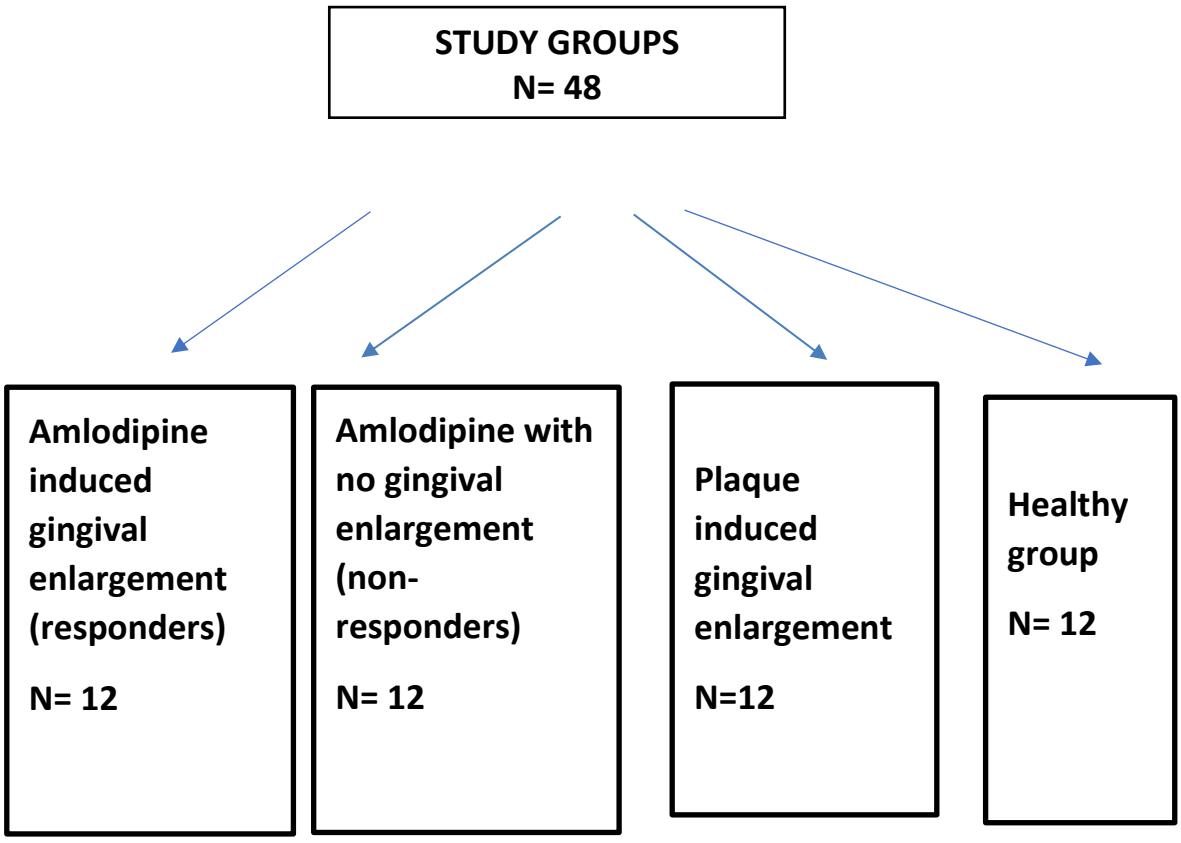
1. Subjects in the age group of 30 - 70 years.
2. Both male and female subjects.

B. EXCLUSION CRITERIA: -

1. Subjects taking any other medications are known to induce gingival overgrowth beside amlodipine / along with amlodipine.

2. Subjects with Periodontal abscess, Necrotizing Periodontal diseases, or periodontitis as a manifestation of systemic disease.
3. Subjects with systemic diseases such as Diabetes mellitus, Rheumatoid Arthritis.
4. Subjects who have received periodontal therapy, within preceding six months.
5. Tobacco in any form and alcoholics.
6. Subjects who have received anti-inflammatory drugs and antibiotics within the preceding 3 months.
7. Subjects having immunological disorders, and infectious diseases.
8. Subjects with any neoplastic conditions.
9. Pregnant and lactating women.

10. PROJECT IMPLEMENTATION PLAN



INCLUSION CRITERIA: - Along with general inclusion criteria: 1) Subjects taking amlodipine with gingival enlargement. **EXCLUSION CRITERIA**: - Along with general exclusion criteria: 1) subjects with plaque induced gingival enlargement.	**INCLUSION CRITERIA**: - Along with general inclusion criteria: 1)Subjects on amlodipine **EXCLUSION CRITERIA**: - Along with general exclusion criteria: 1) subjects taking amlodipine with gingival enlargement.	**INCLUSION CRITERIA**: - Along with general inclusion criteria: 1) Subjects with plaque induced gingival enlargement. **EXCLUSION CRITERIA**: - Along with general exclusion criteria: 1) drug induced gingival enlargement.	**INCLUSION CRITERIA**: - Along with general inclusion criteria: 1) Subjects with good general health. **EXCLUSION CRITERIA**: - Along with general exclusion criteria: 1.subjects with any gingival enlargement.

METHODS OF COLLECTION OF DATA: -

METHODOLOGY: -

Gingival tissue samples obtained from 48 subjects are divided into four group as follows:

Group I: 12 gingival tissue samples from amlodipine responders obtained during gingivectomy procedure.

Group II: 12 gingival tissue samples from amlodipine non-responders during extraction procedure.

Group III: 12 gingival tissue samples from chronic inflammatory gingival

enlargement subjects during gingivectomy.

Group IV: 12 gingival tissue samples in healthy subjects during crown lengthening / extraction procedure.

Oral hygiene instructions will be given for group I, II and III subjects. Oral prophylaxis will be given to all the subjects participants.

Gingival index (Loe and Silness)[4], plaque index (Silness and Loe), gingival overgrowth index (originally described by Angelopoulous and Goaz and later modified by Miller and Damm)[5] and bleeding on probing, will be measured one day before gingival tissue sample collection. The clinical measurements will be carried out by the same examiner, using UNC-15 periodontal probe. To rule out any bone loss orthopantomogram (OPG) radiograph will be taken.

COLLECTION OF GINGIVAL TISSUE SAMPLES:

Gingival tissue samples will be obtained from the selected volunteer during tooth extraction / surgical crown lengthening procedure /gingivectomy. 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 and in case of periodontal surgery. The specimen therefore will be consisting of gingival margin, the sulcular epithelium and the gingival connective tissues and will be stored in -70^0 C till assay procedure. On the day of the assay, the tissue samples 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 centrifuge to remove debris and insoluble material & will be assayed by Enzyme Linked Immunosorbent Assay (ELISA).

Estimation of IL-17A and p53 will be done by using commercially available ELISA kit and the ELISA procedures will be performed based on the instructions provided by the ELISA kit manufacturers.

11. ETHICS REVIEW:

The study requires intervention to be conducted on human subjects. The gingival tissue samples will be obtained from the subjects during periodontal surgery/ tooth extraction/ crown lengthening procedures.

Ethical clearance is obtained from Institutional Ethical Committee and Review Board, Government Dental College and Research Institute, Bengaluru. Ethical clearance number is No.GDCRI/1EC-ACM(2)/10/2020-21.

12. DATA COLLECTION & STATISTICAL ANALYSIS PLAN -

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

13. EXPECTED OUTCOMES –

The pathogenesis of amlodipine induced gingival enlargement is multifactorial. Studies have shown that inflammation plays the most important role in the induction of apoptosis and proliferation in gingival tissues. The present study is expected to show that the expression of p53 protein in gingival enlargement may suggest that the pathogenesis of gingival enlargement is involved with impaired DNA and also shows that amlodipine might cause an increase expression of IL-17A in gingival tissues, that in-turn responsible for fibrotic changes and EMT.

Thus, the present is conducted to elucidate the combined role of IL 17a and p53 in the pathogenesis of amlodipine induced gingival enlargement due to its interaction with amlodipine and its role in apoptosis and inflammation.

14. LIMITATIONS OF THIS STUDY –

Since the present study is cross-sectional study design, the results obtained should be further confirmed with longitudinal study designs.

15. FUTURE PLANS BASED ON EXPECTED OUTCOMES IF ANY-

Further longitudinal and randomized controlled studies with long term follow up should be carrier out to evaluate the multifactorial pathogenesis of amlodipine induced gingival enlargement.

16. TIMELINES:

18 months

17. INSTITUTIONAL SUPPORT: The basic armamentarium e.g., ELISA reader will be provided from institution.

LIST OF REFERENCES: -

1. Sume SS, Berker E, Ilarslan Y, Ozer Yodel O, Tan C, Goyushov S, Gultekin SE, Tezcan I. Elevated Interleukin-17A expression in amlodipine-induced gingival overgrowth. J Periodontal Res. 2020 Oct;55(5):613-621.

2. Buduneli E, Genel F, Atilla G, Kütükçüler N. Evaluation of p53, bcl-2, and interleukin-15 levels in gingival crevicular fluid of cyclosporin A-treated patients. J Periodontol. 2003 Apr;74(4):506-11.

3. Meng F, Wang K, Aoyama T, Grivennikov SI, Paik Y, Scholten D, Cong M, Iwaisako K, Liu X, Zhang M, Österreicher CH, Stickel F, Ley K, Brenner DA, Kisseleva T. Interleukin-17 signaling in inflammatory, Kupffer cells, and hepatic stellate cells exacerbates liver fibrosis in mice. Gastroenterology. 2012 Sep;143(3):765-776.

4. Oseko F, Yamamoto T, Ichioka H, Adachi T, Nishigaki M, Amemiya T, Kanamura N. Cytokine expression in gingival hyperplasia induced by cyclosporine A in mice. Journal of Oral and Maxillofacial Surgery. 2014 Sep 1;72(9):e97-8.

5. Loe H, Theilade E, Jensen SB. Experimental Gingivitis in Man. *J Periodontol.*1965 ;36:177-87.

6. Miller CS, Damm DD. Incidence of verapamil-induced gingival hyperplasia in a dental population. J Periodontol. 1992 May;63(5):453-6.

Section-C

BIODATA OF THE INVESTIGATORS(S)

1. Name First name(s): Arya Ravindranath
2. Designation: BDS, 2nd year MDS (currently)
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Phone no. :7990472312

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4. Date of Birth: 12/ 05/1995

5. Educational Qualification: Degrees obtained (Begin with Bachelor's Degree)

Degree	Institution	Field(s)	Year
BDS	King George Medical University, Lucknow		2017

6. Research/Training Experience

Duration	Institution	Particulars of work don

7. Important recent publications, with titles and References), including papers In press
8. Financial support received
 a. (* From * Past * Present * Pending *)

This information must be given, otherwise the application will be returned. In case no financial assistance has been received, nil should be stated. Indicate titles of the projects and reference number, if available, for grants