Original Article

DOI: 10.7860/JCDR/2015/14492.6388

Dentistry Section

Angiotensin II Levels in Gingival Tissues from Healthy Individuals, Patients with Nifedipine Induced Gingival Overgrowth and Non Responders on Nifedipine

ANITHA BALAJI[1], THODUR MADAPUSI BALAJI[2], SURESH RANGA RAO[3]

ABSTRACT

Context: The Renin Angiotensin system has been implicated in the pathogenesis of Drug Induced Gingival Overgrowth (DIGO), a fibrotic condition, caused by Phenytoin, Nifedipine and Cyclosporine.

Aim: This study quantified Angiotensin II levels in gingival tissue samples obtained from healthy individuals, patients on Nifedipine manifesting/not manifesting drug induced gingival overgrowth.

Materials and Methods: Gingival tissue samples were obtained from healthy individuals (n=24), patients on nifidipine manifesting gingival overgrowth (n= 18) and patients on nifidipine not manifesting gingival overgrowth (n=8). Angiotensin II levels were estimated in the samples using a commercially available ELISA kit.

Results: Angiotensin II levels were significantly elevated in patients on Nifedipine manifesting gingival overgrowth compared to the other 2 groups ($p<0.01$).

Conclusion: The results of the study give an insight into the role played by Angiotensin II in the pathogenesis of drug induced gingival overgrowth.

Keywords: DIGO, Fibrosis, Hypertension

INTRODUCTION

Hypertension is a cardiovascular disease that denotes elevated blood pressure. It is regarded a lifestyle disorder that affects multiple organ systems. Recently, a global increase in the incidence of hypertension has been observed. The National health and nutritional examination survey performed in 2011 revealed that the age-adjusted prevalence of hypertension among US adults aged 18 and over was 29.1% in 2011–2012, similar to the prevalence in 2009–2010 [1]. With a phenomenal increase in global incidence and prevalence of hypertension, it is anticipated that there will be a consistent increase in patient intake of Nifedipine, a calcium channel blocker used for the pharmacological management of hypertension. Nifedipine causes gingival enlargement as an adverse effect. This condition is also called Drug Induced Gingival Overgrowth (DIGO). Other drugs that are implicated in causing DIGO include the antiepileptic agent phenytoin and the immunosuppressant Cyclosporine.

Hassel et al., proposed that the enlargement of the gingiva in DIGO occurs due to an increase in production of extracellular matrix with an unchanged ratio of cell to matrix [2]. Risk factors such as age [3], poor oral hygiene [4], genetic predisposition [5], duration and dosage of drug intake [6] have been known to influence the relationship between drugs and the gingival tissues. Patients who respond to the drugs untowardly develop DIGO (responders) and those who do not manifest DIGO are termed non responders.

Hormones are also known to play an important role in DIGO as evidenced by studies which have shown increased testosterone levels [7] and glucocoticoid receptors [8] in phenytoin induced gingival overgrowth. Recently, the role of the Renin Angiotensin system in the pathogenesis of fibrotic conditions has been researched. Angiotensin II, the effector peptide of the Renin Angiotensin system has been found to induce constriction, hypertrophy and proliferation of various cell types [9]. Angiotensin II has been implicated to cause renal fibrosis through stimulation and upregulation of growth factors [10].

A fully operational Renin Angiotensin system has been found to exist in the rat gingiva by Santos and co-workers [11]. A study by Ohuchi and co-workers has shown the existence of receptors for Angiotensin II namely AT1 and AT2 in rabbit gingival fibroblasts [12]. A previous study by the same group showed the upregualtion of angiotensin II production in cultured guinea pig gingival fibroblasts on addition of Phenytoin and Nifedipine [13]. With the available background information, we performed this study to measure angiotensin II levels in healthy individuals, Nifedipine induced gingival overgrowth patients and patients on Nifedipine not manifesting drug induced gingival overgrowth (non responders) to understand the role of Angiotensin II in the pathogenesis of DIGO.

MATERIALS AND METHODS

The study was performed in the Department of Periodontology of the Faculty of Dental Sciences, Sri Ramachandra University, Chennai, India. Ethical approval was obtained from the Institutional ethical committee of Sri Ramachandra University prior to the start of the study. Informed consent was obtained from all the study participants prior to recruiting them into the study. The study comprised of 3 groups *viz.* Group 1: healthy individuals (n=24), Group 2: patients on Nifedipine manifesting DIGO (n=18) and Group 3: patients on Nifedipine with no DIGO (non responders) (n=8). The inclusion criteria for group 1 were the presence of clinically healthy gingiva with absence of attachment loss and bleeding on probing and also the absence of any systemic diseases. The inclusion criteria for group 2 were hypertensive patients on Nifedipine 5mg once daily dose taken atleast for a minimum of 3 months with no other systemic disease who manifested the clinical signs of gingival overgrowth as described by Angelopolous and Goaz [14]. The gingival overgrowth severity index given by Pernu HE et al., [15] was used in the study based on which patients were assessed and gingival tissue samples were obtained from sites with an overgrowth severity score of 2 to achieve uniformity in sampling. The inclusion criteria for Group 3 were hypertensive patients on Nifedipine 5mg once daily dose taken atleast for a minimum of 3 months with no other systemic disease who did not manifest the clinical signs of gingival overgrowth. The exclusion criteria for the study were pregnancy, lactation, antibiotic and NSAID use in the

last 6 months and intake of any other drugs that cause gingival overgrowth.

Medical fitness was obtained from the physician of the participants prior to collecting the gingival tissue samples. All necessary precautions were taken as hypertensive patients were recruited in the study. Plain lignocaine 2% without adrenaline was used as the local anaesthetic agent for the surgery. Gingival tissue samples were collected from healthy individuals and non responders during surgical crown lengthening done for prosthodontic purpose. Gingival tissue samples were obtained from patients on Nifedipine with DIGO during surgical gingivectomy. All participants underwent professional scaling and plaque control measures prior to the surgery to prepare the tissues and avoid excessive bleeding during surgery and to eliminate the confounding effects of dental plaque induced inflammation on Angiotensin levels. The gingival tissue samples were rinsed in saline after collection, homogenised by mechanical maceration in phosphate buffered saline and centrifuged at 10000 g for 15 minutes. The supernatant obtained was stored in -80 degrees Celsius till further processing. A commercially available ELISA kit (Cayman chemicals Inc) was used to estimate Angiotensin II in the samples. After sample purification, ELISA procedure was performed according to the instructions provided in the kit and the results were finally obtained by subjecting the 96 well microtitre plate to the ELISA reader at 414 nm. The results were compared with standards and blank wells provided in the kit.

STATISTICAL ANALYSIS

The results were statistically analysed using SPSS software version 16. Multivariate analysis was performed using Kruskal Wallis test. To find the significance difference between the bivariate samples in independent groups the Mann-Whitney U test was used. In both the above statistical tools the probability value <0.05 is considered as significant and probability value of <0.01 is considered highly significant

RESULTS

The study comprised of 3 groups *viz.* Group 1: healthy individuals (n=24), Group 2 :patients on Nifedipine manifesting DIGO (n=18) and Group 3 : patients on Nifedipine with no DIGO (non responders) (n=8). The demographic details of the study participants are depicted in [Table/Fig-1].

The mean Angiotensin II, levels in the gingival homogenate samples of the study groups in depicted in [Table/Fig-2].

Multivariate analysis by Kruskal Wallis test revealed a statistically significant difference in Angiotensin II levels between the 3 groups with a p-value of .0001 denoting high statistical significance [Table/Fig-3].

Bivariate analysis by Mann Whitney U-test revealed statistically significant difference in angiotensin II values between the group pairs namely group 1 and group 2, group 2 and group 3, group 3 and group 1 with p-values of .001 denoting high statistical significance. The Angiotensin II values were highest in Group 2 [Table/Fig-4].

DISCUSSION

Though a relationship between Angiotensin II and Gingival overgrowth has been demonstrated in animals, to our knowledge this may be the first study to show a correlation between the two, in humans in vivo. Angiotensin II, the effector peptide of the Renin Angiotensin system is generated by cleavage of Angiotensinogen to Angiotensin I and finally to Angiotensin II. Angiotensin converting enzyme is responsible for the conversion mentioned above. However, enzymes such as chymase which is a product of mast cells could also mediate conversion of Angiotensinogen to Angiotensin I and Angiotensin II. Angiotensin II is regarded a proinflammatory, procoagulant and profibrotic cytokine and is known to exert its effects on target cells through 2 receptors AT1 and AT2.

Group Number	Group Description	Total Number of Subjects	Number of Male Subjects	Number of Female Subjects	Average Age in Years Depicted as Mean ±SD
1.	Healthy Individuals	24	16	8	22.87 ± 2.99
2.	Nifedipine Induced Gingival Overgrowth	18	6	12	51.13 ± 4.61
3.	Non Responders on Nifedipine	8	4	4	54.12 ± 2.45

[Table/Fig-1]: Demographic details of the study groups

Group	Mean Angiotensin Levels In Pcmol/Ml Depicted As Mean ± Sd
Healthy individuals	8.26±4.20
Nifedipine induced gingival overgrowth	13.45±3.27
Non-responders on Nifedipine	0.63±0.33

[Table/Fig-2]: Mean angiotensin levels in gingival tissue homogenates of healthy individuals, Nifedipine induced gingival overgrowth patients and non-responders on Nifedipine

Group	Mean	SD	p-Values
Healthy individuals	8.26	4.21	0.001**
Nifedipine induced gingival overgrowth	13.45	3.27	0.001**
Non-responders on Nifedipine	0.63	0.33	0.001**

[Table/Fig-3]: Multivariate analysis by Kruskal-Wallis test
*Significant at p<0.05, ** Highly significant at p < 0.01

Groups	Z-Values	p-Values
Nifedipine induced gingival overgrowth Non-responders on Nifedipine	4.01	0.0001**
Non-responders on Nifedipine Healthy individuals	3.66	0.0001**
Nifedipine induced gingival overgrowth Healthy individuals	4.18	0.0001**

[Table/Fig-4]: Bivariate analysis by Mann Whitney U-test
*Significant at p<0.05, ** Highly significant at p< 0.01

The significant increase in Angiotensin II levels in the Nifedipine induced gingival overgrowth patients compared to healthy individuals and non responders as shown by our study, confirms the role of Renin Angiotensin system in DIGO. A study by Santos and co-workers has shown a fully operating RAS in rat gingiva [11] and recently we have shown the presence of Angiotensin Converting Enzyme in gingival fibroblasts (unpublished data). This fact may rule out the need of mast cell chymase in the gingiva for Angiotensin II generation. Evidence by the studies of Ohuchi and co-workers show the presence of AT1 and AT2 receptors for Angiotensin II in rabbit gingival fibroblasts [12]. In our lab too, we have shown the presence of AT1 and AT2 receptors in cultured human gingival fibroblasts (unpublished data). The presence of high amounts of Angiotensin II in the DIGO samples as shown in our study could lead to receptor mediated effects.

Angiotensin II was previously thought only as a vasoactive agent. But recent evidence suggests its role in fibrosis. A study by Kim and Iwao has reported the role of angiotensin II in myocardial fibrosis [16]. It has also been demonstrated that angiotensin II modulates renal cell growth and extracellular matrix synthesis by upregulating two significant growth factors namely Transforming Growth Factor β and Connective Tissue Growth Factor [10].

Inflammation of the gingiva induced by dental plaque has been regarded a pre requisite for drug induced gingival overgrowth.

Angiotensin II levels could be modulated by inflammation. However in our study, we eliminated the confounding effects of plaque induced inflammation by performing scaling and plaque control measures and re-evaluating the patients gingival status prior to gingival tissue sample collection. Our findings are also supported by the fact that gingival fibroblasts in cultures where the inflammatory millieu cannot be created also respond to the drug administration and have shown molecular changes pertaining to fibrosis. The most probable reason could be the presence of Angiotensin II in the serum added to cultures. The significantly low levels of Angiotensin II in non responders ($p<.01$) suggests that along with the drug intake a high level of Angiotensin II is required to modulate the pathogenesis of DIGO.

LIMITATION OF THE STUDY

The limitation of our study is the fact that we have not assessed the AT1 and AT2 receptor levels in the tissue samples. We are planning future in vivo and in vitro studies to validate the presence of a fully functional Renin Angiotensin system in the human gingiva.

CONCLUSION

In conclusion our study has shown increased levels of Angiotensin II in Nifedipine induced gingival overgrowth. Angiotensin II could modulate the pathogenesis of DIGO similar to renal and myocardial fibrosis. These effects could be mediated through the AT1 and AT2 receptors. If patients with hypertension could be pharmacologically managed with Angiotensin II antagonists like Losartan and Angiotensin receptor blockers, the occurrence of DIGO could be prevented. Clinical studies in this regard could benefit the human populations world wide.

REFERENCES

[1] Nwankwo T, Sug S (Sarah) Yoon RN, Vicki Burt RN, Qiuping G. Hypertension Among Adults in the United States: National Health and Nutrition Examination Survey, 2011–2012. Centers for disease control and prevention NCHS data brief. 2013;No:133.

[2] Hassell TM, Page RC, Lindhe J. Histologic evidence for impaired growth control in diphenylhydantoin gingival overgrowth in man. *Arch Oral Biol*. 1978;23: 381-84.

[3] Daley TD, Wysocki GP, May C. Clinical and pharmacological correlations in Cyclosporine A induced gingival hyperplasia. *Oral Surg Oral Med Oral Pathol Oral Radiol Endodontol*. 1986;62:417-21.

[4] Seymour RA, Heasman PA. Drugs and the Periodontium. *J Clin Periodontol*. 1988;15:11-16.

[5] Cebeci I, Kantarci A, Firatli E, et al. Evaluation of the frequency of HLA determinants in patients with gingival overgrowth induced by Cyclosporine A. *J Clin Periodontol*. 1996;23:737-42.

[6] Margiotta V, Pizzo I, Pizzo G, Barbaro A. Cyclosporine A and Nifedipine induced gingival overgrowth in renal transplant patients: correlations with periodontal and pharmacological parameters, and HLA antigens. *J Oral Pathol Med*. 1996;25:128-34.

[7] Sooriyamoorthy M, Harvey W, Gower DB. The use of human gingival fibroblasts in culture for studying the effects of Phenytoin on testosterone metabolism. *Arch Oral Biol*. 1988;33:353-59.

[8] Rao SR, Dhar SC. Increased glucocorticoid receptors in Phenytoin induced gingival overgrowth. *Biochem Int*. 1991;24:669-76.

[9] Mehta PK, Griendling KK. Angiotensin II cell signalling. Physiological and Pathological effects in Cardiovascular system. *Am J Physiol Cell Physiol*. 2007;292:82-97.

[10] Kagamai S, Border WA, Miller DE, Noble NA. Angiotensin II stimulates extracellular matrix synthesis through induction of transforming growth factor beta in rat glomerular mesangial cells. *J Clin Invest*. 1994;93:2431-37.

[11] Santos CF, Akashi AE, Dionisio TJ, et al. Characterisation of a local Renin Angiotensin system in rat gingival tissue. *J Periodontol*. 2009;80:130-39.

[12] Ohuchi N, Hayashi K, Koike K, et al. Pharmacological properties of Angiotensin II receptors in cultured rabbit gingival fibroblasts *Comp Biochem Physiol Toxicol Pharmacol*. 2004;137:281-89.

[13] Ohuchi N, Koike K, Sano M, et al. Proliferative effects of Angiotensin II and Endothelin 1 on guinea pig gingival fibroblasts in culture. *Comp Biochem Physiol Toxicol Pharmacol.* 2002;132:451-60.

[14] Angelopoulous AP, Goaz PW. Incidence of diphenylhydantoin gingival hyperplasia. *Oral Surg Oral Med Oral Pathol*. 1972;34:898-906.

[15] Pernu HE, Pernu LM, Knuuttila ML, Huttunen KR. Gingival overgrowth among renal transplant recipients and uremic patients. *Nephrol Dial Transplant*. 1993;8(11):1254-58.

[16] Kim S, Iwao H. Molecular and cellular mechanisms of Angiotensin II mediated cardiovascular and renal diseases. *Pharmacol Rev*. 2000;52:11-34.

PARTICULARS OF CONTRIBUTORS:

1. Professor, Department of Periodontology, Shree Balaji Dental College and Hospital, Pallikaranai, Chennai, Tamil Nadu, India.
2. Associate Professor, Department of Periodontology, Faculty of Dental Sciences, Sri Ramachandra University, Porur, Chennai, Tamil Nadu, India.
3. Professor and Head, Department of Periodontology, Faculty of Dental Sciences, Sri Ramachandra University, Porur, Chennai, Tamil Nadu, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Suresh Ranga Rao,
Professor and Head, Department of Periodontology, Faculty of Dental Sciences,
Sri Ramachandra University, Porur, Chennai-600116, Tamil Nadu, India.
E-mail: chennaidentist@gmail.com

FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Apr 16, 2015**
Date of Peer Review: **Jun 03, 2015**
Date of Acceptance: **Jul 08, 2015**
Date of Publishing: **Aug 01, 2015**