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Clinico Pathological Correlation of Salivary TNF-Alpha Level in Hypertensive Patients under Treatment

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ABSTRACT

Background: TNF- alpha is the key pro-inflammatory cytokines and plays a vital role in the progress of hypertension. At present, hypertensive patients are under treatment with Angiotensin-converting enzyme (ACE) inhibitors, calcium channel blockers, or diuretics. Recent literature revealed that TNF-alpha levels were suppressed in chronic renal failure and congestive heart failure after treatment with amlodipine and captopril.
Aim: The aim of this current study is to determine whether there is any correlation of levels of salivary TNF- alpha level in hypertension along with the action of Calcium channel blockers and ACE inhibitors.
Materials and methods: A cross-sectional pilot study in 10 patients with confirmed hypertension. They were categorized into two study groups namely ACE inhibitors and Calcium channel blockers based on their treatment. The salivary samples were collected in an Eppendorf tube passively and samples were then centrifuged for 5minutes at 5000g to get the supernatant. Salivary TNF-alpha was analysed in patients by ELISA kits. The obtained results were subjected to statistical analysis using an independent t-test. Test values of $p<0.05$ were considered statistically significant.
Results: Salivary TNF- alpha level in hypertensive patients treated with ACE inhibitors was significantly found to be 57.1 ± 13.26 (ng/l) while treated with Calcium channel blockers was significantly found to be 16.12 ± 2.161 (ng/l).
Conclusion: Salivary TNF- alpha in hypertensive patients treated with ACE inhibitors was significantly higher when compared to hypertensive patients treated with Calcium channel blockers. From these findings, Calcium channel blocker-treated hypertensive patients suppressed TNF- alpha production, which leads to possible prevention of the progression of hypertension. Further, the use of easily accessible salivary TNF- alpha could be a potential biomarker and therapeutic target for the disease.

Key words: Salivary TNF- alpha, Hypertension, Calcium channel blockers, ACE inhibitors, ELISA assay, Innovative technique

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INTRODUCTION

Tumor necrosis factor (TNF-alpha) is a pleiotropic polypeptide that functions as a proinflammatory cytokine and regulates cell differentiation, proliferation, and death, as well as inflammation. Immune cells, mostly T-lymphocytes, produce it, but it can also emerge from white blood cells, vascular endothelial cells, and renal tubular epithelial and mesangial cells [1]. As its name implies, cytotoxic or apoptotic effects of TNF-α enhance protection against tumor formation. The tumour necrosis factor gene polymorphism, which is found in the short arm of the sixth chromosome, has been linked to hyperuricemia, transferable disorders, metabolic syndromes, and strokes [2]. Recent research suggests that histological features of angiotensin II (ANG II)-dependent hypertension, such as the development of vascular smooth muscle cells and the proliferation and infiltration of monocytes in the kidney, are linked to increased TNF-α production [3], TNF-α is elevated in numerous hypertension experimental models: Dahl salt-sensitive rats [4] and the nitric oxide (NO)-a deficient model of hypertension induced by treatment with NO synthase (NOS) inhibitor nitro-l-arginine (l-NAME) [5]. It induces necrosis when injected into sites undergoing delayed-type hypersensitivity responses to the mycobacterial antigen. It is either undetectable or found in low concentrations in the circulation under normal conditions, but its production and secretion increase in the setting of inflammation [6].

Hypertension is a complex multifactorial disorder with genetic, environmental, and demographic factors

contributing to blood pressure variation. In the UK, the National Institute for Health and Clinical Excellence (NICE) defines High Blood pressure, also known as hypertension, as a clinic blood pressure of 140/90 mmHg or higher confirmed by a subsequent ambulatory blood pressure monitoring daytime average of 135/85 mmHg or higher [7]. The causes of hypertension are known to be secondary to conditions such as pheochromocytoma, primary hyperaldosteronism (Conn's syndrome), Cushing's syndrome (excessive glucocorticoids), renal diseases, or drug induced. The associated complications are left ventricular hypertrophy, diastolic dysfunction, congestive heart failure, cerebral thrombosis/ haemorrhage, hypertensive encephalopathy, microalbuminuria, carotid artery stiffening, arterial aneurysms, and dissections, and retinopathy [8]. Currently, around a third of people with hypertension are undiagnosed and of those diagnosed, around half are not taking antihypertensive medications. The World Health Organisation (WHO) predicts that high blood pressure kills at least nine million people worldwide per year, either directly or indirectly [9]. This is important because preventing hypertension lowers the chance of developing cardiovascular disease in the future.

New approaches are therefore needed to enhance monitoring, diagnosis, and management of increased blood pressure in the population. So, breaking away from the conventional cuff-based assessment of blood pressure, the widespread accessibility of smartphones and mobile health applications also provides the opportunities for the pervasive control of blood pressure [10]. Salivary composition depends on systemic and local factors. Thus, it may reflect pathophysiological pathways relevant to the progress of both systemic and local disease [11]. In this aspect, salivary TNF- alpha is used as a diagnostic to aid the assessment of periodontal disease, endocrinology, neurology, oncology, and infectious diseases [12]. By reviewing the previous literature, we are interested in choosing hypertensive patients undergoing treatment to analyse changes in salivary TNF-alpha level due to its role in the pathophysiology of Hypertension. Our team has extensive knowledge and research experience that has translated into high quality publications [13-32]. The aim of this study is to determine the correlation between the levels of salivary TNF- alpha in Hypertension along with the action of calcium channel blockers and ACE inhibitors.

MATERIALS AND METHODS

An in vivo cross-sectional study on saliva samples of various hypertensive patients treated with Calcium channel blocker and ACE inhibitors for the estimation of salivary TNF- alpha levels. The study was non-invasive and easy to perform without much inconvenience to patients. However, the sample size was limited. Before the initiation of the study, clearance was obtained by the Scientific Review Board with Ethical approval number IHEC/SDC/BDS/1929/01. The samples were obtained from patients who came to the clinics of Saveetha Dental College and Hospitals. The number of samples collected was 10 (n=10). The samples were collected in an unbiased manner using randomized sampling. Validation was done by an expert pathologist.

Criteria for selection of study subjects

Patients with clinically confirmed Hypertension and are treated with Calcium channel blockers and ACE inhibitors. They were categorized into two study groups namely, Calcium channel blockers and ACE inhibitors. It was also ensured that patients with systemic comorbidities or terminally ill patients were not taken up for the study.

All the subjects included in the study belonged to the same ethnic group of South India (Dravidian population). Informed consent was obtained from the study subjects for inclusion in the study and it was also ensured that the subject's anonymity was maintained. All the participants completed a questionnaire covering medical, residential, and occupational history.

Sample collection

10 saliva samples were collected from patients with clinically confirmed hypertension and who are all under treatment with Calcium channel blockers and ACE inhibitors. Unstimulated saliva from the patients was collected in Eppendorf's for a volume of 1ml. Then it was stored at -200 Celsius. During the procedure, it was thawed and centrifuged. Samples were collected during the time frame between November 2020 to January 2021.

Principle of the test

ELISA is based on the competitive binding technique in which the TNF- alpha present in the sample competes with a fixed amount of horseradish peroxide (HRP)-labelled TNF- alpha on a human monoclonal antibody. Standards and samples are pipetted into the wells and TNF- alpha present in a sample is bound to the wells by the immobilized antibody. The wells are washed and a biotinylated anti-human TNF- alpha antibody was added. After washing away the unbound biotinylated antibody, HRP conjugated streptavidin is pipetted to the wells. The wells were washed again, a TMB substrate solution is added to the wells and color develops in proportion to the amount of TNF- alpha bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

Reagent preparation

All reagents and samples were brought to room temperature (18-25°C) before use. Also, Assay Diluent B (Item E) should be diluted to 5-fold with deionized or distilled water before use. For dilution of sample Assay Diluent, A (Item D) should be used for dilution of serum and plasma samples. The suggested dilution for normal serum/plasma is 2-20-fold. For the preparation of the standard, a vial of Item C was briefly spun. 400 µL of Assay Diluent A (for serum/ plasma samples) was added into Item C vial to prepare 50ng/ml standard. The powder was dissolved thoroughly by a gentle mix.

15 µL TNF- alpha standard (50 ng/ml) was added from the vial of Item C, into a tube with 485 µL Assay Diluent A or 1X Assay Diluent B to prepare a 1,500 pg/ml standard solution. 400 µL Assay Diluent A or 1X Assay Diluent B was pipetted into each tube. 1,500 pg/ml standard solution was used to produce a dilution series (shown below). Each tube was mixed thoroughly before the next transfer. Assay Diluent A or 1X Assay Diluent B served as the zero standards (0 pg/ml). If the Wash Concentrate (20X) (Item B) contained visible crystals, it was warmed to room temperature and mixed gently until they dissolved.

20 ml of Wash Buffer Concentrate was diluted into deionized or distilled water to yield 400 ml of 1X Wash Buffer. Detection Antibody vial (Item F) was briefly spun before use. 100 µL of 1X Assay Diluent B (Item E) was added into the vial to prepare a detection antibody concentrate. This was then pipetted up and down to mix gently (the concentrate can be stored at 4°C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1X Assay Diluent B (Item E) and used in relevant prior steps. The HRP-TNF- alpha concentrate vial (Item G) was briefly spun and pipetted up and down to mix gently before use, as precipitates may form during storage. HRP- TNF-alpha concentrate should be diluted 200-fold with 1X Assay Diluent B (Item E).

Assay procedure

All reagents and samples were brought to room temperature (18-25°C) before use. It is recommended that all standards and samples be run at least in duplicate. Removable 8-well strips were labelled as appropriate for the experiment. 100 µl of each standard and sample was added into appropriate wells. These wells were then covered and incubated for 2.5 hours at room temperature with gentle shaking. The solution was discarded and washed 4 times with 1X wash solution. Each well was filled and washed with Wash Buffer (300 µl) using a Pipette. Complete removal of the liquid at each step is essential for good performance. After the last wash, any remaining Wash Buffer was removed by aspirating or decanting. The plate was inverted and blotted with clean paper towels. 100 µl of 1X prepared biotinylated antibody was added to each well. This was then incubated for 1 hour with gentle shaking. The solution was discarded, and the wash was repeated. 100 µL of prepared Streptavidin solution was added to each well. This was then incubated for 45 minutes at room temperature with gentle shaking. The solution was discarded, and the wash repeated. 100 µL of TMB One-Step Substrate Reagent (Item H) was added to each well and incubated for 30 minutes at room temperature in the dark with gentle shaking. 50 µl of Stop Solution (Item I) was added to each well and read at 450 nm immediately.

Calculation of results

The mean absorbance was calculated for each set of duplicate standards, controls, and samples, and the average zero standard optical density was subtracted. The standard curve was plotted using Sigma plot software, with standard concentration on the x-axis and absorbance on the y- axis. The best-fit straight line was drawn through the standard points.

Typical data

A standard curve was run with each assay.

Sensitivity

The minimum detectable dose of Human RAGE was determined to be 3pg/ml. The minimum detectable dose is defined as the analyte concentration resulting in an absorbance that is 2 standard deviations higher than that of the blank (diluents buffer).

Statistical analysis

Statistical analysis was performed using SPSS software. An Independent t-test was done to compare the results. Dependent variables include Hypertension while independent variables include age and sex.

RESULTS

Demographic data

The study included a total of n=10 participants who were divided into two groups. Group I consists of hypertension patients treated with Calcium channel blocker n=5, Group II consists of hypertension patients treated with ACE inhibitors n=5.

Statistics

Statistical analysis was performed using SPSS (IBM SPSS Statistics for Windows Version 23.0, Armonk, NY: IBM Corp. Released 2015). An Independent t-test was done to compare the groups and $p<0.05$ was considered significant and $p>0.05$ was considered not significant.

Prevalence of salivary TNF-alpha levels among the groups

Salivary TNF-alpha in hypertensive patients treated with calcium channel blocker

Among the 5 samples analysed, all 5 showed the presence of TNF-alpha in salivary samples. The prevalence of salivary TNF-alpha among Group I was 100% with a minimum value of 16.12 ng/dl and a maximum value of 2.161 ng/dl.

Salivary TNF-alpha in hypertensive patients treated with ACE inhibitors

Among the 5 samples analysed, all 5 showed the presence of TNF-alpha in salivary samples. The prevalence of salivary TNF-alpha among Group II was 100% with a minimum value of 57.1 ng/dl and a maximum value of 13.26 ng/dl.

Comparison of prevalence of TNF-alpha among salivary samples of hypertensive patients treated with Calcium channel blockers and ACE inhibitors

The comparison between the groups shows statistical significance; a higher prevalence of salivary TNF-alpha was found among Hypertensive patients treated with ACE inhibitors when compared to those treated with Calcium channel blockers (Figure 1 and Table 1).

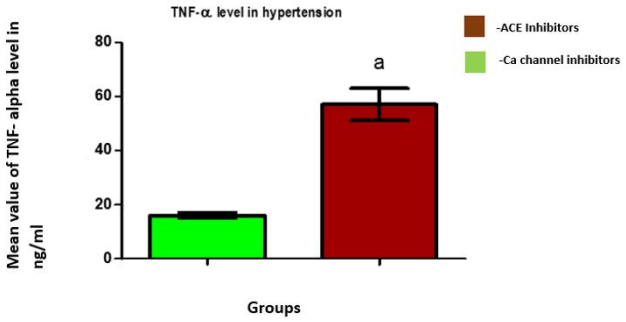

Figure 1: Bar graph showing salivary TNF- alpha levels in ACE inhibitors and Ca channel inhibitors. The X-axis represents the study group and Y- axis represents concentrations of salivary TNF-alpha. Green denotes Ca channel inhibitors and red denotes ACE inhibitors. The graph indicates that there is a significant increase in the salivary levels of TNF-alpha for patients using ACE inhibitors (p<0.05).

Table 1: Table showing mean ± S.D of three observations at the levels p<0.05.

Group	Mean	Std. Deviation	Std. Error	P Value
Calcium channel blocker	16.12	2.161	0.9664	<0.05
ACE inhibitors	57.1	13.26	5.931	

DISCUSSION

Various cytokines play an important role in inflammatory and carcinogenic processes. It is secreted by activated monocytes or macrophages in response to inflammatory factors such as lipopolysaccharide (LPS) [33]. These macrophages represent a major defense system against invasion of the host by a range of microorganisms including that of bacteria, viruses, fungi, and protozoa. Amongst, TNF-alpha is a multifunctional cytokine that assists in the normal growth and homeostasis of many organs, as well as mediating inflammation, immune response, and apoptosis [34]. It induces constriction of blood vessels and hence the control of blood pressure by stimulating the lining of blood vessels to secrete vasoactive substances in the blood vessels via a paracrine or autocrine pattern. In addition, TNF, being an endogenous pyrogen, can induce fever, apoptotic cell death, cachexia, and inhibit tumorigenesis, viral replication as well as respond to sepsis via IL-1 and IL-6-producing cells. This macrophage-derived TNFα is now implicated in a number of diseases, including rheumatoid arthritis, inflammatory bowel disease, psoriatic arthritis, ankylosing spondylitis, juvenile chronic arthritis and sepsis [35].

In this regard, several studies have determined the specific role of TNFα signaling mechanisms, TNF-alpha gene polymorphisms, TNF- alpha antagonist and demyelination disease, Anti- tumour necrosis factor therapy for COVID-19 [36]. Further studies carried on salivary TNF-α levels in chronic periodontitis, which was found to be significantly higher in chronic periodontitis affected individuals than in healthy subjects, but there was no significant correlation with the clinical parameters [37]. Similar studies reported that there was a negative relationship between TNF-α and IL-1α levels with pocket depth and attachment loss [38].

Hypertension is a low-grade inflammatory condition characterized by the presence of various proinflammatory cytokines, in which TNF- alpha acts especially on the vasculature, kidney, and sympathetic nervous system to regulate blood pressure [6]. Various studies investigated the role of serum TNF-alpha in regulation of increased blood pressure. The elevation of TNF- alpha level might result in decreased blood pressure, whereas moderate increases in TNF- alpha have been associated with increased NaCl retention and hypertension. The explanation for these disparate effects is not clear but could simply be due to different concentrations of TNF- alpha within the kidney, physiological status of the subject, or the type of stimulus initiating the inflammatory response [39]. In Controversy to previous study, increased production of TNF- alpha is associated with angiotensin II (ANG II)- dependent hypertension [40].

TNF-alpha inhibition has important therapeutic implications for the treatment of hypertension and associated organ failure. Serum level of TNF- alpha has been used in some studies for monitoring the therapeutic response of Hypertension [41]. According to current innovation, salivary biomarkers analyse an array of proteins, mRNA and DNA for the oral and systemic diseases. This method is considered as a first choice of non-invasive screening approach that can be collected easily for repeated number of times, easy transfer, storage, shipping and affordable. A positive statistical correlation exists between TNF- alpha level in saliva as a serum [42].

Previous studies investigated the inhibition of TNF- alpha in hypertension. TNF- alpha inhibition reduced systolic blood pressure as well as increased survival rate in hypertensive rats with left ventricular hypertrophy [43]. Using infliximab infusion, which is an TNF- alpha inhibitor improves blood pressure as well as endothelial function and reduces arterial stiffness in patients with rheumatoid arthritis [44]. Several researchers have studied the modulation of TNF- alpha production with anti-hypertensive drugs. Amlodipine, a calcium channel blocker, administered at doses of 15mg/ kg and 25 mg/ kg in hypertensive patients and evaluated serum TNF- alpha level which was found to be lower due to suppression of Calcium channel blocker [45]. Similarly, in our study, TNF- alpha level was found to be lower and the values are 16.12 +/- 2.161 (ng/l) in patients treated with Calcium channel blockers.

Also, patients treated with oral administration of captopril, delapril, and cilazapril (ACE inhibitors) significantly inhibited the serum TNF- alpha production. At a posttranscriptional level, ACE-inhibitors suppress IL-1 and TNF synthesis and might therefore influence cytokine-mediated cell growth [46]. Another study suggested that the use of ACE-inhibitors is associated with lower plasma TNF-alpha [47]. This contrasts with our study, TNF- alpha significantly elevated in hypertensive patients treated with ACE inhibitors. The limitations of this study include a limited sample size. Also, we didn't categorize hypertensive patients based on stages of hypertension. In further studies with increased sample size and saliva is a perfect medium useful for monitoring health and disease state.

CONCLUSION

Within the limitations of this study, salivary TNF- alpha level in hypertensive patients treated with ACE inhibitors was significantly higher when compared to hypertensive patients treated with Calcium channel blockers. From these findings, Calcium channel blocker-treated hypertensive patients suppressed TNF- alpha production, which leads to possible prevention of the progression of hypertension. Further, the use of easily accessible salivary TNF- alpha could be a potential biomarker and therapeutic target for the disease.

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CONFLICT OF INTEREST

The authors would like to declare no conflict of interest in the present study.

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REFERENCES

1. Ramseyer VD, Garvin JL. Tumor necrosis factor-α: Regulation of renal function and blood pressure. Am J Physiol Renal Physiol 2013; 304:F1231–42.
2. Qidwai T, Khan F. Tumour necrosis factor gene polymorphism and disease prevalence. Scandinavian J Immunol 2011; 74:522–547.
3. Guzik TJ. Role of the T cell in the genesis of angiotensin II induced hypertension and vascular dysfunction. J Exp Med 2007; 204:2449–24460.
4. Huang B. Renal tumor necrosis factor α contributes to hypertension in dahl salt-sensitive rats. Scientific Reports 2016; 6:21960.
5. Shahid M, Francis J, Matrougui K, et al. Involvement of tumor necrosis factor-alpha in natriuretic response to systemic infusion of nitric oxide synthase inhibitor in anesthetized mice. Am J Physiol Renal Physiol 2010; 299:F217–24.
6. Mehaffey E, Majid DSA. Tumor necrosis factor-α, kidney function, and hypertension. Am J Physiol Renal Physiol 2017; 313:F1005–8.
7. Elliott WJ. Hypertension: Management of hypertension in adults in primary care: partial update. Yearbook of Cardiology 2007; 2007: 5–7.
8. Lin FY, Devereux RB. Hypertensive heart failure. Echocardiography Heart Failure 2012; 79–98.
9. Kitt J. New approaches in hypertension management: A review of current and developing technologies and their potential impact on hypertension care. Current Hypertension Reports 2019; 21:44.
10. Chandrasekhar A. Smartphone-based blood pressure monitoring via the oscillometric finger-pressing method. Science Translational Med 2018; 10.
11. Iorgulescu G. Saliva between normal and pathological. Important factors in determining systemic and oral health. J Med Life 2009; 2:303–307.
12. Wei F, Wong DT. Point-of-care platforms for salivary diagnostics. Chinese J Dent Res 2012; 15:7-15.
13. Anita R, Paramasivam A, Priyadharsini JV, et al. The m6A readers YTHDF1 and YTHDF3 aberrations associated with metastasis and predict poor prognosis in breast cancer patients. Am J Cancer Res 2020; 10:2546–54.
14. Jayaseelan VP, Paramasivam A. Emerging role of NET inhibitors in cardiovascular diseases. Hypertens Res 2020; 43:1459–61.

15. Sivakumar S, Smiline Girija AS, Vijayashree Priyadharsini J. Evaluation of the inhibitory effect of caffeic acid and gallic acid on tetR and tetM efflux pumps mediating tetracycline resistance in Streptococcus sp., using computational approach. J King Saud University Sci 2020; 32:904–909.
16. Smiline Girija AS. Delineating the immuno-dominant antigenic vaccine peptides against gacs-sensor kinase in Acinetobacter baumannii: An in silico Investigational Approach. Front Microbiol 2020; 11:2078.
17. Iswarya Jaisankar A, Smiline Girija AS, Gunasekaran S, et al. Molecular characterisation of csgA gene among ESBL strains of A. baumannii and targeting with essential oil compounds from Azadirachta indica. J King Saud University Sci 2020; 32:3380–3387.
18. Girija ASS. Fox3+ CD25+ CD4+ T-regulatory cells may transform the nCoV's final destiny to CNS! J Med Virol 2020.
19. Jayaseelan VP, Ramesh A, Arumugam P. Breast cancer and DDT: putative interactions, associated gene alterations, and molecular pathways. Environ Sci Pollut Res Int 2021; 28:27162–27173.
20. Arumugam P, George R, Jayaseelan VP. Aberrations of m6A regulators are associated with tumorigenesis and metastasis in head and neck squamous cell carcinoma. Arch Oral Biol 2021; 122:105030.
21. Kumar SP, Girija AS, Priyadharsini JV. Targeting NM23-H1-mediated inhibition of tumour metastasis in viral hepatitis with bioactive compounds from ganoderma lucidum: A computational study. Indian J Pharm Sci 2020; 82:300-305.
22. Girija SA, Priyadharsini JV, Paramasivam A. Prevalence of carbapenem-hydrolyzing OXA-type β-lactamases among Acinetobacter baumannii in patients with severe urinary tract infection. Acta Microbiol Immunol Hung 2019; 67:49–55.
23. Priyadharsini JV, Paramasivam A. RNA editors: Key regulators of viral response in cancer patients. Epigenomics 2021; 13:165–167.
24. Mathivadani V, Smiline AS, Priyadharsini JV. Targeting epstein-barr virus nuclear antigen 1 (EBNA-1) with Murraya koengii bio-compounds: An in-silico approach. Acta Virol 2020; 64:93–99.
25. Girija AS, Priyadharsini JV. Prevalence of Acb and non-Acb complex in elderly population with urinary tract infection (UTI). Acta Clin Belg 2021; 76:106–112.
26. Anchana SR, Girija SAS, Gunasekaran S, et al. Detection of csgA gene in carbapenem-resistant Acinetobacter baumannii strains and targeting with Ocimum sanctum biocompounds. Iran J Basic Med Sci 2021; 24:690–698.
27. Girija ASS, Shoba G, Priyadharsini JV. Accessing the T-Cell and B-Cell immuno-dominant peptides from A. baumannii biofilm associated protein (bap) as vaccine candidates: A computational approach. Int J Pept Res Ther 2021; 27:37–45.
28. Arvind P TR, Jain RK. Skeletally anchored forsus fatigue resistant device for correction of Class II malocclusions-A systematic review and meta-analysis. Orthod Craniofac Res 2021; 24:52–61.
29. Venugopal A, Vaid N, Bowman SJ. Outstanding, yet redundant? After all, you may be another Choluteca Bridge! Semin Orthod 2021; 27:53–56.
30. Ramadurai N, Gurunathan D, Samuel AV, et al. Effectiveness of 2% articaine as an anesthetic agent in children: Randomized controlled trial. Clin Oral Investig 2019; 23:3543–50.
31. Varghese SS, Ramesh A, Veeraiyan DN. Blended module-based teaching in biostatistics and research methodology: A retrospective study with postgraduate dental students. J Dent Educ 2019; 83:445–450.
32. Mathew MG, Samuel SR, Soni AJ, et al. Evaluation of adhesion of Streptococcus mutans, plaque accumulation on zirconia and stainless steel crowns, and surrounding gingival inflammation in primary molars: Randomized controlled trial. Clin Oral Investigations 2020; 24:3275–3280.
33. Brozovich FV, Nicholson CJ, Degen CV, et al. Mechanisms of vascular smooth muscle contraction and the basis for pharmacologic treatment of smooth muscle disorders. Pharmacol Rev 2016; 68:476-532.
34. Parameswaran N, Patial S. Tumor necrosis factor-α signaling in macrophages. Critical Reviews 2010; 20.
35. Subramaniam K, Tymms K, Shadbolt B, et al. Spondyloarthropathy in inflammatory bowel disease patients on TNF inhibitors. Int Med J 2015; 45:1154–1160.
36. Feldmann M, Maini RN, Woody JN, et al. Trials of anti-tumour necrosis factor therapy for COVID-19 are urgently needed. Lancet 2020; 395:1407–1409.
37. Varghese SS, Thomas H, Jayakumar ND, et al. Estimation of salivary tumor necrosis factor-alpha in chronic and aggressive periodontitis patients. Contemp Clin Dent 2015; 6:S152–6.
38. Eivazi M, Falahi N, Eivazi N, et al. The effect of scaling and root planning on salivary TNF-α and IL-1α concentrations in patients with chronic periodontitis. Open Dent J 2017; 11.
39. Jayaram A, Deer E, Amaral LM, et al. The role of tumor necrosis factor in triggering activation of natural killer cell, multi-organ mitochondrial dysfunction and hypertension during pregnancy. Pregnancy Hypertension 2021; 24:65–72.

40. Faria APD. Effects of tnf-alpha inhibiton on hemodynamic parameters and biomarkers in resistant hypertension. J Hypertension 2018; 36:e48–9.
41. Li G, Wu W, Zhang X, et al. Serum levels of tumor necrosis factor alpha in patients with IgA nephropathy are closely associated with disease severity. BMC Nephrol 2018; 19:326.
42. Agho ET. Salivary inflammatory biomarkers and glycated haemoglobin among patients with type 2 diabetic mellitus. BMC Oral Health 2021; 21:101.
43. Filho AG. Infliximab prevents increased systolic blood pressure and upregulates the AKT/eNOS pathway in the aorta of spontaneously hypertensive rats. Eur J Pharmacol 2013; 700:201–209.
44. Abdelrahman AM. Effect of infliximab and tocilizumab on fructose-induced hyperinsulinemia and hypertension in rats. Biomed Pharmacotherapy 2018; 105:182–186.
45. Mendis S. Calcium channel blockers and alpha-blockers in hypertension: what is the evidence? Ceylon Med J 2014; 45:152.
46. Schindler R. Angiotensin-converting-enzyme inhibitors suppress synthesis of tumour necrosis factor and interleukin 1 by human peripheral blood mononuclear cells. Cytokine 1995; 7:526–533.
47. Stenvinkel P, Andersson P, Wang T, et al. Do ACE-inhibitors suppress tumour necrosis factor-alpha production in advanced chronic renal failure? J Intern Med 1999; 246:503–507.