How to Cite:
Arya, A., Sreekanth, S., Tirupathi, S., Kumar, N., Saikrishna, S., Yeso, A., Meena, M., & Afnan, L. (2022). Clinical periodontal parameters and sub-gingival bacterial profile in chronic periodontitis patients with and without chronic kidney disease (CKD): A cross-sectional observational study. *International Journal of Health Sciences*, *6*(S6), 1424–1436. https://doi.org/10.53730/ijhs.v6nS6.9967

Clinical periodontal parameters and sub-gingival bacterial profile in chronic periodontitis patients with and without chronic kidney disease (CKD): A cross-sectional observational study

Aishwarya Arya
Assistant professor, Department of Periodontics, Mallareddy dental college and Hospital, Suraram x roads, Hyderabad, Telangana

Sreekanth
Professor and Head, Department of Periodontics, Mallareddy dental college and Hospital, Suraram x roads, Hyderabad, Telangana

Sunnypriyatham Tirupathi
Assistant professor, Department of Pediatric and preventive dentistry, Dr.D.Y.Patil Dental College and Hospital, Dr.D.Y.Patil Vidyapeeth, Pimpri, Pune, Maharasthra, India
*Corresponding author email: dr.priyatham@gmail.com

Naresh Kumar
Senior Lecturer, Department of Periodontics, Tirumala institute of Dental Sciences, Nizamabad, Telangana

Saikrishna
Assistant professor, Department of Prosthodontics, Sri Balaji dental college and hospital, Moinabad, Hyderabad, Telangana.

Alekhya Yeso
Consultant Oral medicine and Radiologist, Clove Hospital, Hyderabad, Telangana

Meena
Consultant Oral medicine and Radiologist, Clove Hospital, Hyderabad, Telangana

Lamea Afnan
Assistant professor, Department of Public health dentistry, Dr.D.Y. Patil Dental College and Hospital, Dr.D.Y.Patil Vidyapeeth, Pimpri, Pune, Maharasthra, India

***Abstract*---**Objectives: To assess the severity of periodontitis and to determine the sub-gingival microbial profile in patients with and without chronic kidney disease (CKD). Methods: A total of 40 patients (18 females and 22 males) were selected for the study and were divided into two groups of twenty each. The test group (Group I) consisted of 20 patients (8 females and 12 males) chronic periodontitis with chronic kidney disease, and control group (Group II) had 20 patients(10 males and 10 females) chronic periodontitis without chronic kidney disease (CKD).Clinical periodontal parameters such as PPD(pocket probing depth),CAL(clinical attachment loss),BOP (bleeding on probing) and Sub-Gingival Bacterial profile. Results: Results of this study suggests that clinical parameters such as PPD (pocket probing depth), CAL(clinical attachment loss), BOP (bleeding on probing) and sub-gingival bacterial profile belonging to red complex are significantly more in patients with CKD. Conclusion: CP presented more severe clinical parameters and is associated with increased frequency of trponema denticolae, tanerella forsythia, porphyromonas gingivalis in patients with CKD.

***Keywords*---**bacterial profile, chronic kidney disease, periodontitis, oral health.

Introduction

Periodontitis is a relatively common inflammatory disease that contributes to the loss of connective tissue attachment and the supporting bone around teeth. The reciprocal action of pathogenic bacteria, host's immune and inflammatory responses together contribute to the tissue destruction in periodontal disease progression.[1] Bacterial virulence factors released by periodontal pathogens result in triggered release of biologic mediators from host tissue cells such as Proteinases, Cytokines and Prostaglandins which are the inflammatory mediators produced as a part of host response, which contribute to host tissue destruction.[2] Chronic kidney disease (CKD) is a generic term and includes a variety of renal pathologies that determine the progressive loss of glomerular filtration rate (GFR). A plethora of uremia-specific risk factors coexist and they contribute to the increased cardiovascular risk in CKD population. Poor oral health, advanced age, diabetes mellitus, are few under-rated risk factors for development of CKD. Significant association between poor oral health and systemic complications in CKD such atherosclerosis, infections, protein-energy wasting (PEW) is reported in literature.[3] Bacterial products (antigens, endotoxins) and inflammatory cytokines that spread through the circulatory system induces systemic responses in periodontitis patients. Inflamed periodontal tissue can act as a potential source for chronic systemic inflammation, CP is a potential risk factor for CKD.[4] Compromised oral condition of CKD patients can be attributed to metabolic, uremic, endocrinal and immunological imbalances. Dialysis patients often present with one or more oral symptoms such as xerostomia, uremic odor, tongue coating, taste disturbances, mucosal inflammation, mucosal petechia/ecchymosis, oral ulceration, or enamel hypoplasia. Xerostomia can result in gingival inflammation, dysguesia, dysphagia, speech difficulty, denture

retention, mastication, sore mouth. Retrograde parotitis is a common finding in CKD patients as a result from a combination of direct and indirect factors such as dehydration, and mouth breathing ,direct gland involvement, drug related side-effects. Patients with renal failure often complain of an ammonia-like bad odour due to high urea content in saliva which gets converted to ammonia. Higher concentration of urea and phosphate levels in saliva results in increased dental calculus accumulation. Increased levels of plaque accumulation are reported in HD patients. The poor oral hygiene and gingival inflammation can be due to compromised oral care in ESRD patients. Chronic periodontitis was significantly more severe among HD and CKD patients as compared to normal individuals. [5-9] Most of the studies published in the literature provide evidence for increased prevalence of periodontal disease in patients with renal disease, especially in established ESRD.[10-13] However studies pertaining to determination of prevalence of periodontal disease in pre-dialysis stage are scarce. Hence, the aim of the present study is to assess the severity of periodontitis and to determine the sub-gingival microbial profile in patients with and without chronic kidney disease (CKD). To assess the severity of periodontitis and to determine the sub-gingival microbial profile in patients with and without chronic kidney disease (CKD).

Materials and Methods

This cross-sectional observational study was undertaken in the out-patient department of Periodontics, Mamata Dental College & Department of General Medicine, Mamata Medical College, Khammam in collaboration with the Department of Microbiology and Research, Maratha Mandal NGH Institute of Dental Sciences and Research centre, Belgaum, Karnataka from June to September 2017. Before the commencement of the study, an informed consent was obtained from the participating subjects. The study protocol approved by the institutional review committee for human research. Protocol no: (MDC_T_D158806025) Patients aged above 35 years with chronic kidney disease in Pre- dialysis stage with more than ten teeth were included in the study. Subjects who had undergone periodontal therapy in last six months, patients who have end stage renal disease undergoing haemodialysis , HIV patients, pregnant women, patients with cardiovascular diseases, uncontrolled diabetes, smoking habit were excluded from the study.

Criteria for selection of chronic periodontitis patient: Chronic periodontitis patients were selected based on the criteria by Page and Eke (2007). According to the definition, patients with ≥2 sites with interproximal CAL ≥ 4mm, not on the same tooth or ≥2 sites with interproximal PD ≥4mm, not on the same tooth were diagnosed as chronic periodontitis. [14] Systemic medical evaluation : Laboratory tests for blood such as serum creatinine, blood count, blood glucose was evaluated. If the fasting blood glucose levels were 126 mg/dl or 200 mg/dL, 2 h after an overload of 75 g of oral glucose then they are considered as having diabetes mellitus. Urine anaylasis was also carried out to look for abnormal elements and sediments. The diagnosis and stage of the CKD were established as recommended by US National Kidney Foundation: Signs of kidney damage (such as albuminuria or glomerular hematuria or structural abnormalities as noted on imaging studies) or a GFR less than 60 mL/min per 1.73 m^2 for more than three

months.[15] Other parameters such as blood pressure and heart rate is also measured in all the subjects.

Dental evaluation : A blinded investigator evaluated periodontal parameters such as Bleeding On Probing (BOP), Clinical Attachment Level (CAL) and Pocket Probing Depth (PPD).Sub-Gingival bacterial profile in chronic periodontitis patients with and without chronic kidney disease (CKD). figure-1 Pocket Probing Depth (PPD):The depth of the pocket was measured using UNC-15 periodontal probe which was inserted parallel to the long axis of the tooth gently, until resistance was felt and readings were recorded to the nearest millimetre from the gingival margin to the base of the pocket.[16] Clinical Attachment Level (CAL) : Clinical attachment loss was evaluated similarly as mentioned above with UNC-15 periodontal probe. The probe was inserted parallel to the long axis of the tooth. The readings were recorded to the nearest millimetre from the cementoenamel junction to the base of the pocket. When recession of the gingival was present, the CAL was calculated by adding the distance from cementoenamel junction (CEJ) to the margin of gingiva. When the gingival margin was coronal to CEJ, the CAL was calculated by subtracting the distance from the gingival margin to CEJ. When the gingival margin was at the CEJ, no calculations were needed since the probing depth and the clinical attachment level were equal.[16] figure-1 Bleeding On Probing (BOP) : Bleeding on probing is evaluated using modified sulcus bleeding index (Scores 0-0 No bleeding when a periodontal probe is passed along the gingival margin; Score-1 Isolated bleeding spots visible; Score-2 Blood forms a confluent red line on margin;Score-3 Heavy or profuse bleeding).[17] Sub-Gingival bacterial profile : The subgingival plaque sample was collected by using sterile curette. The sterile curette was first inserted deep into the selected site and the sub-gingival plaque was then removed with a single pull stroke and was transferred to a vial of transport media containing Tris-HCL-EDTA buffer (TE buffer). These vials were stored at 4°C and transported within 48hrs for Polymerase Chain Reaction (PCR) analysis

Multiplex Polymerase Chain Reaction (PCR) analysis : Sub gingival sample collected is subjected to centrifugation at 10000rpm for 5minutes.Sample is separated into Sediment and supernatant. Supernatant is discarded. Sediment is washed with TE buffer for 4 times and subjected to centrifugation 3 times at 10000rpm for 5minutes.Supernatant discarded again. 50µL Lysis buffer I and 50µl Lysis buffer II to sediment and incubated with 100 µg/ml of Proteinase K (chromous biotech®) for 2 hours at 60°C, followed by boiling for 10 min at 95 0C Supernatant stored at -20 0C till processed. PCR was performed using specific primers(16S rRNA) for DNA encoding of species P. gingivalis, T. forsythia, and A. actinomycetemcomitans. PCR requires components such as DNA template and Primers. The upstream primers for the three organisms. DNA polymerase (Taq polymerase) which copies the region to be amplified.dNTPs from which DNA polymer builds new DNA. Buffer which provides a suitable environment for DNA polymerase. The reagents were added to the sample in laminar air flow. The enzyme was added last and mixed by pipetting. DNA amplification was carried out in a thermocycler (palm- cycler®). After adding the loading dye, the molecular sizes of the amplicons were determined. The frequency of positive sites was evaluated for each microbiota. figure-2

Statistical analyses: The results are presented as means ± standard deviation (SD) for numerical variables and as absolute and relative frequencies for categorical variables. Clinical and laboratory characteristics and periodontal indexes were compared among the groups using the chi-squared test for ordinal data and independent t test for intergroup comparison of scale data.

Results

A total of 40 patients (18 females and 22 males)were selected for the study and were divided into two groups of twenty each. The test group (Group I) consisted of 20 patients (8 females and 12 males) chronic periodontitis with chronic kidney disease, and control group (Group II) had 20 patients(10 males and 10 females) chronic periodontitis without chronic kidney disease. Mean age is 48.65 in group I and 48.05 in group II. Table 1 Pocket Probing Depth (PPD): In Group I the mean± standard deviation PPD was 6.55± 1.05 mm, and in Group II the mean± standard deviation PPD was 5.95± 0.75 mm. there is significantly higher pocket probing depth in Group I in comparison to Group II and the difference is significant (P-value 0.045). Table 2

Clinical Attachment Level (CAL) : In Group I the mean± standard deviation CAL was 3.85± 1.04 mm, and in Group II the mean± standard deviation CAL was 3.30± 0.47 mm. there is significantly higher clinical attachment loss in Group I in comparision to Group II and the difference is significant (P-value 0.038). Table 2 Bleeding On Probing (BOP): In Group I the mean± standard deviation BOP was 2.32± 0.25, and in Group II the mean± standard deviation BOP was 1.72± 0.21. There is significantly higher Bleeding On Probing in Group I in comparision to Group II and the difference is significant (P-value 0.001). Table 2 Sub-Gingival bacterial profile : On PCR anaylasis bacterial species belonging to red complex such as trponema denticolae, tanerella forsythia, porphyromonas gingivalis were found in significantly higher concentration in subgingival biofilm collected from Group I than in Group II (P-value 0.027). Table 3

Discussion

Periodontitis refers to the inflammatory process that results in the tissue destruction surrounding the teeth in response to bacteria, dental plaque accumulation on the teeth which results in inflammatory host response. Plaque components can induce infiltrate of inflammatory cells, including polymorphonuclear leukocytes (PMNs), lymphocytes and macrophages. Microbial components such as lipopolysaccharide (LPS), activate macrophages to synthesize and secrete a variety of pro-inflammatory components such as interleukin-1 (IL-1); prostaglandin E2 (PGE2); and tumor necrosis factor-alpha (TNF-alpha); and other hydrolytic enzymes which result in release of collagenolytic enzymes such as metalloproteinases (MMPs) cause periodontal tissue breakdown. [1] Within the past 10 years, many studies have been published indicating a positive or negative relationship between periodontal disease and various systemic disorders and diseases. Patients diagnosed with periodontal disease present higher risk due to a compromised immunity. [19,20] CKD is defined as either kidney damage (i.e. pathological abnormalities or markers that represent damage) or an eGFR <60 ml/min/m^2 for more than 3 months.[21] The CKD comprise a cluster of diverse

renal diseases, including dialysis and kidney transplanted patients.[22] According to the most recent and revised classification from the KDIGO (Kidney Disease: Improving Global Outcomes) Conference Report, CKD is characterized by both estimated rate of glomerular filtration (eGFR) and albuminuria stages.[23]

However, eGFR is considered the best outcome to measure the level of kidney function and to determine the stage of CKD. [2] It has been suggested that different forms of acute and chronic inflammatory processes can stimulate an inflammatory response in the kidneys, leading to CKD. [24,25] CKD is associated with many risk factors such as periodontitis, diabetes mellitus, smoking and age.· Furthermore, it has also been suggested that periodontitis could be considered a non-traditional risk for CKD due to the following factors: (a) systemic inflammation burden caused by periodontal inflammation (and its locally produced inflammatory mediator such as IL-1, IL-6, PGE2 and TNF-α and (b) the presence of bacteria and their products in the bloodstream. Recent evidence has shown that patients with periodontitis may have elevated levels of C-reactive protein and, consequently a mild acute-phase systemic inflammatory response when compared with healthy subjects. [26] Paraskevas and co-workers based on their systematic review, reported that the link between chronic systemic inflammation and CKD is measured by the levels of C-reactive protein, and periodontitis as a source of "permanent inflammation" could contribute to CKD.[27]

With respect to the presence of bacteria in the bloodstream, circulating periodontal bacteria could lead to kidney endothelium damage.[28,29] It seems biologically plausible that such an association could constitute a source of systemic inflammatory burden. [30,31] Moreover, in many cases, the aetiology of kidney disease remains unknown and cannot be attributed due to usual causes, such as diabetes mellitus, hypertension, diabetes mellitus, pyelonephritis, glomerulonephritis, collagen vascular disease, nephrosclerosis, polycystic kidney disease. [32,33] Anand and co-workers reported the prevalence of 1 in 12 individuals living in two of India's largest cities have evidence of CKD, with features that put them at high risk for adverse outcomes.[34] Joseph and co-workers in their study evaluated the prevalence and severity of periodontal disease among the patients with renal disease and compared to that of healthy controls and concluded that among patients with renal disease a greater prevalence and severity of periodontal disease is reported. [35] Castillo and co-workers compared periodontal pathogens using (PCR) in sub-gingival plaque samples isolated from CKD patients on hemodialysis with those from individuals without systemic disease. The authors found no significant correlation between both parameters. [36]

Bastos and co-workers conducted a study with 47 CKD (25 patients with pre-dialysis & 22 patients with Renal replacement therapy) patients to assess the association between severity of the CKD and periodontal pathogens and reported that CP severity was more in patients with CKD. [13] Takeuchi et al study evaluated the sub-gingival microbial profile in patients with CKD. Authors reported that Prevotella nigrescens, Candida albicans, Tannerella forsythia, were more frequent in CKD patients than in the controls. [12] Despite the relative evidence of a possible association between periodontal disease and CKD, the link between these two diseases is not completely established.[37] Thus, the present cross-sectional study was designed to assess the severity of periodontal disease and analysis of

subgingival microbial flora in chronic periodontitis patients with and without CKD. Clinical parameters measured in the present study were Probing Pocket Depth (PPD), Clinical Attachment Level (CAL). Subgingival plaque sample was collected by using sterile curette for microbial analysis. For the detection of periodontal pathogens in the subgingival plaque sample multiplex PCR was used. The organisms assessed were P.gingivalis, A.actinomycetemcomitans, T.forsythia, T.denticola, P.intermedia.

Physiological and Biochemical characteristics

The main systolic and diastolic blood pressure were higher in CKD patients who are on pre-dialysis than in healthy subjects with chronic periodontitis. The GFR was higher in healthy subjects than in patients with CKD who are on pre-dialysis. The results of this study are consistent with the study of Bastos et al, who displayed a statistically significant increased level of systolic and diastolic blood pressure in CKD patients with CP.[13]

Clinical characteristics

The frequency of sites PD>4mm were significantly lower in chronic periodontitis with that of patients in the pre-dialysis group. The frequency of sites with CAL>4mm was lower in chronic periodontitis than that of pre-dialysis group. In the present study it was found that there was an elevated levels of PPD and CAL which confirms the hypothesis that greater prevalence of PD is severe in CKD patients who are on pre-dialysis than in healthy groups with chronic periodontitis. These findings are in accordance with the studies of Davidovich et al, and Bayatkar et al, who have observed the association between elevated levels of gingival inflammation and periodontal disease in chronic kidney disease patients when compared with healthy subjects with chronic periodontitis.[30] Severe periodontal inflammation in these patients could have also contributed to the level of renal disease burden. The systemic disease burden also have influenced the progression of PD in these patients.

Microbial analysis

In the present study CP was associated with a higher frequency of red complex (Pg, Tf, Td) Aa, Pi in patients with CKD and individuals without CKD. These findings are according to the studies of Bastos et al, and Takeuchi et al. [12,13] The abnormalities of both cellular and humoral immunity and phagocytic activity which have been described in vitro and in vivo has been associated with uremic status may explain the increase frequency of red complex, Aa, Pi compared with and without CKD. Another reason of increase prevalence of red complex bacteria may be because of degradation of oral hygiene in chronic periodontitis patients with renal disease. The present study is being a cross-sectional in design, does not establish a cause and effect relationship and did not consider the educational levels of the patients and their socio-economic status, which might effect the periodontal status. In the present study comorbidities, such as malabsorption, that could cause Hypoalbuminaemia have not been recorded. Samples were collected from a single tooth site and not from all sites of the mouth. Within the limits of the present study it can be stated that CKD patients tend to have a high

risk of periodontal disease. Severity of CP is more and is associated with increased P.gingivalis, T.Denticola, T.forsythia, A. actinomycetemcomitans and P. intermedia in patients with CKD. In pre-dialysis chronic kidney disease patients regular evaluation of periodontal tissue status may slow down the progression of the renal disease by reducing inflammatory burden. Therefore, more cross-sectional and case control studies allocating patients into sub groups according to the extension, severity and type of periodontitis will allow more accurate evaluation.

Conclusion

Within the limitation of the present study the following conclusions can be drawn from the results: The present study provides evidence for a greater prevalence and severity of periodontal disease among pre-dialytic patients with renal disease. Periodontal pathogenic bacteria were more frequent in patients with chronic kidney disease than in patients without chronic kidney disease.

Clinical Relevance
Scientific rationale for study

There is a need to evaluate and assess the clinical periodontal parameters and sub-gingival bacterial profile in chronic periodontitis patients with and without chronic kidney disease (CKD) so that treatment parameters such as medication for specific bacterial profiles, and also the frequency of intervention can be adjusted.

Principal findings

Chronic periodontitis patients with chronic kidney disease (CKD) with increased levels of red complex bacteria such as tanerella forsythia, treponema denticolae and porphyromonas gingivalis than in chronic periodontitis patients without chronic kidney disease. Clinical periodontal parameters such as pocket probing depth (PPD), clinical attachment loss (CAL), bleeding on probing (BOP) were more in chronic periodontitis patients with chronic kidney disease.

Practical implications

Chronic kidney disease patients are more prone to complications such as increased severity of periodontal disease and increased periodontal pathogenic bacteria. The clinician should aim to evaluate and increase the frequency of periodontal care and increase the frequency of periodontal status evaluation and also customise the drugs according to the bacterial profile identified.

References

1. Barbato L, Francioni E, Bianchi M, Mascitelli E, Marco LB, Tonelli DP. Periodontitis and bone metabolism. Clin Cases Miner Bone Metab.2015;12:174-177.

2. Needleman I, Garcia R, Gkranias N, Kirkwood KL, Kocher T, Iorio AD, et al. Mean annual attachment, bone level, and tooth loss: A systematic review. J Clin Periodontol.2018;45:S112-S129.
3. Wahid A, Chaudhry S, Ehsan A, Butt S, Ali Khan A. Bidirectional relationship between chronic kidney disease & periodontal disease. Pak J Med Sci.2013;29:211-215.
4. Borawski J, Wilczyńska-Borawska M, Stokowska W, Myśliwiec M. The periodontal status of pre-dialysis chronic kidney disease and maintenance dialysis patients. Nephrol Dial Transplant.2007;22:457-464.
5. Souza CR, Libério SA, Guerra RN, Monteiro S, Silveira EJ, Pereira AL. [assessment of periodontal condition of kidney patients in hemodialysis]. Rev Assoc Med Bras.2005;51:285-289.
6. Craig RG. Interactions between chronic renal disease and periodontal disease. Oral Dis.2008; 14:1-7.
7. Klassen JT, Krasko BM. The dental health status of dialysis patients. J Can Dent Assoc.2002; 68: 34-38.
8. Duran I, Erdemir EO. Periodontal treatment needs of patients with renal disease receiving haemodialysis. Int Dent J.2004;54: 274-278.
9. Chen LP, Chiang CK, Chan CP, Hung KY, Huang CS. Does periodontitis reflect inflammation and malnutrition status in hemodialysis patients? Am J Kidney Dis.2006;47:815-822.
10. Thorman R, Neovius M, Hylander B. Clinical findings in oral health during progression of chronic kidney disease to end-stage renal disease in a swedish population. Scand J Urol Nephrol.2009; 43:154-159.
11. Rahmati MA, Craig RG, Homel P, Kaysen GA, Levin NW. Serum markers of periodontal disease status and inflammation in hemodialysis patients. Am J Kidney Dis.2002;40:983-989.
12. Takeuchi Y, Ishikawa H, Inada M, Shinozuka O, Umeda M, Yamazaki T, et al. Study of the oral microbial flora in patients with renal disease. Nephrology.2007;12:182-190.
13. Bastos JA, Diniz CG, Bastos MG, Vilela EM, Silva VL, Chaoubah A, et al. Identification of periodontal pathogens and severity of periodontitis in patients with and without chronic kidney disease. Arch Oral Biol.2011;56:804-811.
14. Page RC, Eke PI. Case definitions for use in population-based surveillance of periodontitis. J Periodontol.2007;78:1387-1399.
15. K/doqi clinical practice guidelines for chronic kidney disease: Evaluation, classification, and stratification. Am J Kidney Dis.2002;39:S1-266.
16. Syed SA, Svanberg M, Svanberg G. The predominant cultivable dental plaque flora of beagle dogs with gingivitis. J Periodontal Res.1980;15:123-136.
17. Newbrun E. Indices to measure gingival bleeding. J Periodontol.1996;67:555-561.
18. Sorsa T, Ingman T, Suomalainen K, Haapasalo M, Konttinen YT, Lindy O, et al. Identification of proteases from periodontopathogenic bacteria as activators of latent human neutrophil and fibroblast-type interstitial collagenases. Infect Immun.1992;60:4491-1495.
19. Prabhu A, Michalowicz BS, Mathur A. Detection of local and systemic cytokines in adult periodontitis. J Periodontol.1996;67:515-522.

20. Leivadaros E, van der Velden U, Bizzarro S, ten Heggeler JM, Gerdes VE, Hoek FJ, et al. A pilot study into measurements of markers of atherosclerosis in periodontitis. J Periodontol.2005;76:121-128.
21. Levey AS, Coresh J, Balk E, Kausz AT, Levin A, Steffes MW, et al. National kidney foundation practice guidelines for chronic kidney disease: Evaluation, classification, and stratification. Ann Intern Med.2003;139:137-147.
22. Shoji T, Abe T, Matsuo H, Egusa G, Yamasaki Y, Kashihara N, et al. Chronic kidney disease, dyslipidemia, and atherosclerosis. J Atheroscler Thromb.2012;19:299-315.
23. Levey AS, de Jong PE, Coresh J, Nahas MEl, Astor BC, Matsushita K, et al. The definition, classification, and prognosis of chronic kidney disease: A kdigo controversies conference report. Kidney International.2011;80:17-28.
24. Stenvinkel P. New insights on inflammation in chronic kidney disease-genetic and non-genetic factors. Nephrol Ther.2006; 2:111-119.
25. Kshirsagar AV, Offenbacher S, Moss KL, Barros SP, Beck JD. Antibodies to periodontal organisms are associated with decreased kidney function. The dental atherosclerosis risk in communities study. Blood Purif .2007;25:125-132.
26. Fisher MA, Taylor GW, Papapanou PN, Rahman M, Debanne SM. Clinical and serologic markers of periodontal infection and chronic kidney disease. J Periodontol 2008; 79: 1670-78.
27. Paraskevas S, Huizinga JD, Loos BG. A systematic review and meta-analyses on c-reactive protein in relation to periodontitis. J Clin Periodontol.2008;35:277-290.
28. Brotto RS, Vendramini RC, Brunetti IL, Marcantonio RAC, Ramos APP, Pepato MT. Lack of correlation between periodontitis and renal dysfunction in systemically healthy patients. Eur J Dent.2011; 5: 8-18.
29. Grubbs V, Plantinga LC, Crews DC, Bibbins-Domingo K, Saran R, Heung M, et al. Vulnerable populations and the association between periodontal and chronic kidney disease. Clin J Am Soc Nephrol.2011; 6: 711-717.
30. Davidovich E, Davidovits M, Eidelman E, Schwarz Z, Bimstein E. Pathophysiology, therapy, and oral implications of renal failure in children and adolescents: An update. Pediatr Dent.2005; 27: 98-106.
31. Nylund KM, Meurman JH, Heikkinen AM, Furuholm JO, Ortiz F, Ruokonen HM. Oral health in patients with renal disease: A longitudinal study from predialysis to kidney transplantation. Clin Oral Investig.2018; 22:339-347.
32. Bayraktar G, Kurtulus I, Duraduryan A, Cintan S, Kazancioglu R, Yildiz A, et al. Dental and periodontal findings in hemodialysis patients. Oral Dis.2007; 13:393-397.
33. Shultis WA, Weil EJ, Looker HC, Curtis JM, Shlossman M, Genco RJ, et al. Effect of periodontitis on overt nephropathy and end-stage renal disease in type 2 diabetes. Diabetes Care.2007; 30: 306-311.
34. Anand S, Shivashankar R, Ali MK, Kondal D, Binukumar B, Montez-Rath ME, et al. Prevalence of chronic kidney disease in two major indian cities and projections for associated cardiovascular disease. Kidney international.2015; 88:178-185.
35. Rosamma J, Rajaratnam K, Vivek N. Higher prevalence of periodontal disease among patients with predialytic renal disease. Brazilian Journal of Oral Sciences.2015; 8.

36. Castillo A, Mesa F, Liébana J, García-Martinez O, Ruiz S, García-Valdecasas J, et al. Periodontal and oral microbiological status of an adult population undergoing haemodialysis: A cross-sectional study. Oral Dis 2007; 13: 198-205.
37. Nadeem M, Stephen L, Schubert C, Davids MR. Association between periodontitis and systemic inflammation in patients with end-stage renal disease. SADJ.2009; 64:470-473.
38. Rahman, N. M., Made, S., Usman, A. N., Idris, I., Unde, A., & Bahar, B. (2022). Analysis of understanding of midwife students of midwife care as the impact of online learning. International Journal of Health & Medical Sciences, 5(1), 63-66. https://doi.org/10.21744/ijhms.v5n1.1834
39. Widana, I.K., Dewi, G.A.O.C., Suryasa, W. (2020). Ergonomics approach to improve student concentration on learning process of professional ethics. Journal of Advanced Research in Dynamical and Control Systems, 12(7), 429-445.
40. Widana, I.K., Sumetri, N.W., Sutapa, I.K., Suryasa, W. (2021). Anthropometric measures for better cardiovascular and musculoskeletal health. Computer Applications in Engineering Education, 29(3), 550–561. https://doi.org/10.1002/cae.22202

Table 1
Mean age of study population according to gender in Group I & II

Gender	Group I			Group II		
	N	Mean	Std. Deviation	N	Mean	Std. Deviation
Female	10	47.10	4.977	8	50.00	4.957
Male	10	49.00	5.142	12	47.75	5.463
Total	20	48.05	5.021	20	48.65	5.254

Table 2
Intergroup comparison of clinical parameters (PPD,CAL,BOP) among Group I and II

Parameter	Group	N	Mean	Std. Deviation	Std. Error Mean	mean difference	sig	
Pocket Probing Depth	I	20	6.550	1.0501	.2348	-0.6	0.045*	
Pocket Probing Depth	II	20	5.950	.7592	.1698			
Clinical Attachment Loss	I	20	3.850	1.0400	.2325	-0.55	0.038*	
Clinical								

Attachment Loss	II	20	3.300	.4702	.1051			
Bleeding On Probing	I	20	2.03	3.300	.4702	-0.28	0.001*	
Bleeding On Probing	II	20	1.75	3.850	1.0400			

Table 3
Intergroup comparison of sub-gingival bacterial profile among Group I and II

Bacteria	T.Denticola	T.Denticola	P.Gingivalis	P.Gingivalis	A.a	A.a	P.Intermedia	P.Intermedia	T.Forsythia	T.Forsythia
	Group-I	Group-II	Group-I	Group-II	Group-I	Group-II	Group-I	Group-II	Group-I	Group-II
Absent	6	13	7	14	17	18	16	13	7	14
Present	14	7	13	6	3	2	4	7	13	6
Total	20	20	20	20	20	20	20	20	20	20
Chi-Sq	4.912	4.912	4.912	4.912	0.229	0.229	1.129	1.129	4.912	4.912
P-value	0.027*	0.027*	0.027*	0.027*	0633 NS	0633 NS	0.288 NS	0.288 NS	0.027*	0.027*

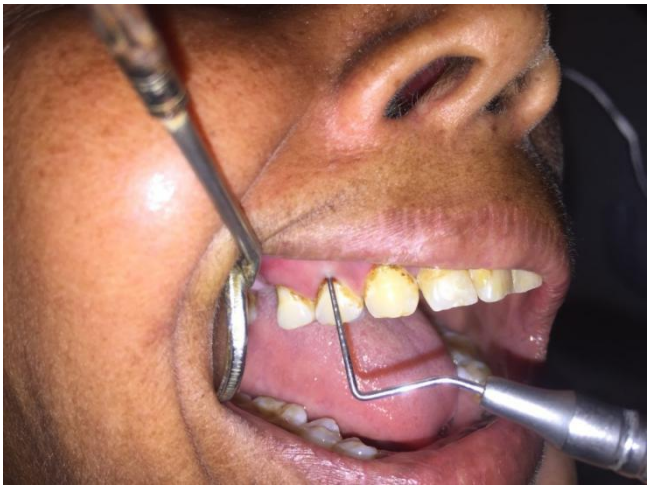

Figure 1. figure showing measurement of probing depth in patients

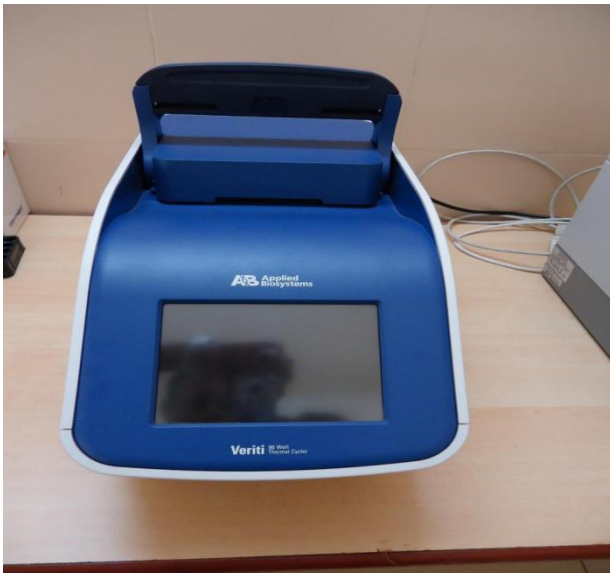

Figure 2. figure showing PCR-Thermal Cycler