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Efficacy of Nonsurgical Periodontal Therapy on Microbiological and Biochemical Variables in Patients with Chronic Renal Disease and Periodontitis.

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Abstract

Context: Periodontitis and Renal disease are chronic inflammatory diseases which share common risk factors. Both the diseases elicit the production of cytokines, prostaglandins, pro-inflammatory mediators which lead to deleterious consequences. Micro-organisms are key players in the pathogenesis of both the conditions.

Aims: This study assessed if scaling and root planing (NSPT) improves the microbiologic and biochemical profile of patients afflicted with both the problems.

Settings and Design: This study was a randomized clinical study which included 40 subjects in the age group ranging from 35-75 years with mean age 58.5 ±8.9

Methods and Material: Twenty subjects having renal disease (Pre-dialysis stage) and chronic periodontitis formed the experimental sample and twenty individuals with only periodontitis formed the controls. All the variables taken were measured in the experimental and control samples before and three months, post NSPT.

Statistical analysis used: Intergroup comparison for continuous normal data was done by unpaired t-test and for continuous non-normal data by wilcoxon rank sum test/mann- whitney U test. Pre and post interpretation for continuous normal data was done by paired t-test and continuous non-normal data by wilcoxon signed ranked test. All P scores<0.05 were interpreted as of significance statistically.

Results: Significant difference was observed at baseline and 3 months postop, pertaining to the gingival Index(GI), *treponema denticola* levels, serum Creatinine and urinary albumin: creatinine Ratio (UACR) between the experimental and control samples.

Conclusions: NSPT proved to be beneficial in improving the GI scores as well as the *treponema denticola*, serum creatinine, and UACR levels.

Keywords: Periodontitis; Chronic renal disease; NSPT; PCR; Blank Standard Test.

Key Messages: NSPT was efficacious in reducing the gingival index scores, and the serum creatinine, urinary albumin: creatinine ratio, *treponema denticola* levels in the experimental samples. Thus this treatment protocol should be readily followed and other new markers should be assessed.

Introduction: Periodontitis is a poly-microbial disease initiated by dental plaque which forms a substrate for the microorganisms to live and thrive. If left untreated the onslaught by the microorganisms as well as the inflammatory products released cause pockets to form with ongoing destruction of the periodontium and bone. Gingival recession could also occur due to the same reason. [1] Renal disease is said to occur when the kidneys are damaged for three, or greater than three months due to structural or functional impairment, with or without a reduction in the glomerular filtration rate (GFR). [2]

Background: It has been observed in many studies that both the diseases are associated with each other and periodontitis (CP) has been implied as a risk factor for Chronic kidney disease (CKD). Nonsurgical Periodontal therapy (NSPT) seems to improve the microcirculation in the kidney thus resulting in better kidney filtration and functioning. [3,4]

Subjects and Methods: A Clinical, Biochemical, and microbiological, study was done to examine the outcome of NSPT on both the diseases. Patients attending the outpatient ward of a tertiary referral care dental hospital as well as visiting a renal and laparoscopic center in Hyderabad formed the samples. The revised Helsinki 2000 declaration guidelines were strictly adhered to during the course of the study. This study was approved by the institutional ethical committee and was also registered (NCT02901743). The samples signed the consent form before commencement of the study. 40 individuals who were aged between 35-75 years with mean age 58.5 ±8.9 (Figure1) participated and were equally divided into two groups.

The study samples were randomly divided by investigator KRR. Clinical parameters were recorded by investigator KMT. All the patients underwent scaling & root planning at baseline by investigator KMT who was blinded to the randomization process

Inclusion Criteria

Group I comprised the test group which included 20 subjects with CKD (abnormal creatinine and GFR rate <60 mL/min/1.73m ^{2}) [5] and CP (with Clinical attachment loss ≥ 4mm, probing depth ≥5mm and bleeding on probing in ≥4 sites in 3 different teeth and a total of 15 teeth remaining in the mouth) [6] and 20 patients diagnosed with CP only. (Group-II- Controls)

Exclusion Criteria

Systemically compromised patients, Smokers and patients with aggressive periodontitis, those individuals under any antibiotic prophylaxis for periodontal therapy for the past six months, Pregnant and lactating women were excluded from participating in the study.

Clinical Parameters: The samples underwent clinical examination wherein the severity of periodontitis was examined using a UNC-15 probe. An occlusal stent was prepared for each patient and grooves marked at the mesial and distal sides of the tooth examined to standardize the angle of insertion of the probe, at baseline and 3 months after NSPT. (Fig 2). Both the gingival (GI-Gingival index) and periodontal condition (PPD-Probing pocket depth, CAL-Clinical attachment level) were recorded at baseline and 3 months after scaling and root planning.

Microbiological Analysis: A sterile gracey curette, was used to obtain the sub-gingival plaque which was taken from pockets with depths ≥ 5mm. The plaque samples were taken at baseline and 3 months after scaling and root planing. The curette was inserted gently to avoid dislocation of sub-gingival plaque. Once the curette met tissue resistance at the bottom of the pocket, sub-gingival sampling was done with one single vertical stroke. Care was taken not to contaminate the plaque sample with

blood. For transport of the sample, the working end of the curette was agitated till the plaque got dispersed in the eppendorf tube containing 10mM of Tris hydrochloride and 1Mm of Ethylene di-amino tetra acetic acid. (Fig 3). 100 μ l of 0.5 M sodium hydroxide was later added and the suspension was preserved at -20°C until DNA extraction. The extraction of DNA was done by Modified Proteinase-K method [7] and the levels of *treponema denticola* and *tanerella forsythia* was estimated using polymerase chain reaction set up(PCR)

PCR Procedure: Levels of *tannerella forsythia* amplicon size 641 bp and *treponema denticola*, amplicon size 316bp were assessed using PCR. (Figure 4). After PCR the samples were stored at 4^0 C and the amplified products were subjected to agarose gel electrophoresis. [8] (Figure 5)

Biochemical analysis: In both groups, serum creatinine & urinary albumin: creatinine ratio was measured preoperatively and 3 months after nonsurgical periodontal therapy. Blood samples were collected (Figure 6) and serum creatinine was estimated by Modified Jaffe's reaction. [9] The urinary albumin creatinine ratio was done by estimation of urinary protein by the turbidimetric method [10] using the Blank standard test (Figure 7)

Outcome measures: The microbiological and biochemical values comprised the primary outcome. The secondary outcome measures assessed were the clinical parameters.

Statistical analysis: Substituting the values for primary outcome measure with power at 80% and level of significance 5%, 20 patients per group was analyzed to be sufficient. The comparison between groups for continuous normal data was done by unpaired t-test and for continuous non-normal data by wilcoxon rank sum test/mann -whitney U test. Pre and post differences for continuous normal data was done by paired t-test and continuous non-normal data by wilcoxon signed ranked test. A value of P <0.05 for the variables measured was considered to be of significance statistically.

Results

Clinical Variables: Though there was a reduction in the probing depth after NSPT in both the groups, when an intergroup comparison was made the results were not of statistical significance. Pertaining to the CAL when an intragroup comparison was made there was an improvement in both groups, however intergroup comparison did not yield significant results. The GI showed statistically significant results in the test when compared to the control group both pre (P value <0.0001) and post operatively (P value 0.043). (Table 1)

Microbiological parameters: The levels of *T.denticola* in the test group reduced after NSPT when compared to the control group (P value 0.021), however Intergroup comparison for the parameter *T. forsythia*, did not yield results. (Table 2)

Biochemical Assessment: Intergroup comparison for serum creatinine, showed better improvement in the test group 3 months postop (P value<0.0001). Urinary albumin creatinine ratio also, was found to have improved postoperatively in the test group (P value<0.0001), when compared to the control group. (Table 3)

Discussion: Periodontitis a globally rampant disease, is caused due to the accumulation of plaque on the tooth surfaces which in turn aids in the adhesion and colonisation of microorganisms. The microorganisms in turn release inflammatory products which are detrimental to the host. The host response if favourable will ward off the microbial insult, failing which the disease progresses and becomes severe. [11]

Periodontitis causes the systemic spread of microorganisms, antigens, endotoxins and inflammatory mediators.Thus the inflammatory response to the disease

can lead to various systemic ailments, one amongst which is chronic renal disease. [12]

A person who has Chronic kidney disease (CKD) will experience ongoing deficit in the functioning of the organ which would culminate in end stage renal disease, wherein the individual would require dialysis or a kidney transplant to improve his life expectancy. Therefore, CKD is associated with poor prognosis. [13]

Harun Akar et al observed that the oral hygiene is poor in patients with renal disease. This may cause inflammation, infection and atherosclerosis which may worsen the condition. Hence maintaining good oral health is mandatory and should be monitored both by the nephrologist and the periodontist. [14]

S. M. Parkar, C. G. Ajithkrishnan, in a study on 304 subjects who were stratified into 152 receiving dialysis who formed the test group and 152 healthy subjects, were the controls,the oral hygiene index simplified (OHIS) and the community periodontal Index were made use of to examine the periodontal status. It was observed that periodontitis was more severe in the test group (P<0.001), when compared to the controls. They concluded that preventive programs should be actively undertaken in patients on dialysis. [15]

It has been observed by researchers that periodontal treatment causes a reduction of inflammation, thus improving the endothelial function. Periodontal treatment may also have an impact on the kidney microcirculation with a subsequent more effective filtration [16]

Other researchers have stated that periodontal treatment may result systemically in a two way manifestation. Short term, periodontal treatment may cause acute trauma resulting in an increase in the inflammatory markers [17] However, the effects of long term periodontal treatment show a decrease in the levels of these markers, suggesting the beneficial effects of periodontal therapy. [18]

Chambrone L et al systematically reviewed the evidence of an association between CKD and CP and also the effect of scaling and root planing on the estimated glomerular filtration rate. Two independent reviewers who searched the literature concluded that there was consistent evidence to support the close association between both the diseases. Moreover, they stated that periodontal treatment had a positive effect on the GFR. [19]

According to this study, the gingival index showed marked improvement after NSPT in both control and test groups (P value0.043) which was statistically significant. Although the other clinical variables did not yield significant results.

There is a disruption of the host homeostasis by the microorganisms in periodontitis and the severity of disease occurring depends on the putativeness of the organisms as well as the genetic makeup of the host. [20] The red complex species [21] have been often associated with cardiovascular disease [22], stroke, preterm low birth weight infants and diabetes mellitus [23]

T. denticola is not seen in the gingival crevicular fluid of healthy individuals, but on the contrary is detected in periodontal pockets. This organism responds to a wide array of chemo attractants, and modifies the genetic framework of the host to rapidly multiply and thrive in periodontal pockets. [24]

Periodontitis was associated with CKD according to a study done by the Atherosclerosis Risk in Communities (ARIC), who further in another study reiterated that high levels of antibodies to *Porphyromonas gingivalis, Treponema denticola, and Aggregatibacter actinomycetemcommitans* were observed in patients with CKD. [25]

In this study both the control and test group, showed a definitive reduction of *Treponema Denticola (*after 3 months follow- up period) following non- surgical

periodontal therapy, (P value 0.021). Though there was a reduction of *T. Forsythia* after NSPT, the results were not statistically significant in both the groups. This observation coincides with another study, [26] wherein the authors remarked that both the age of the patient as well as *T. Forsythia* are independent risk factors for periodontal disease in patients with CKD.

Murthy and other researchers stated that acute injury to the kidneys is characterized by a rapid fall in the glomerular filtration rate. They also emphasized that GFR can be monitored by assessing the serum creatinine concentration as well as the calculated creatinine clearance. [27]

R. Nayak et al studied 24hour urine protein excretion in patients with CKD. The sample size for the study was 100 patients with stage 3 or 4 of renal disease. The daytime random urine sample was taken to measure the protein to creatinine ratio. The accuracy of spot urine protein creatinine ratio (SpUr-PCR) was examined. SpUr-PCR predicted 24- hour urinary protein excretion with good accuracy and there was a good agreement between the markers of proteinuria. [28]

In this study, there was a marked improvement in serum creatinine levels in the test group after NSPT (P value<0.0001). The albumin: creatinine ratio also showed marked improvement in the test group after NSPT (P value<0.0001) which is in accordance with the previous studies

Shortcomings of the study: The sample size was small, a larger sample size could have validated the role of NSPT better, in improving the outcome of both the diseases.

Conclusion

In this study NSPT showed a marked reduction in the gingival Index as well as in microbial load of *Treponema Denticola* in both the groups. There was an improvement in the serum creatinine level and urinary albumin: creatinine ratio in the experimental group, however biochemical parameters in the control group, did not show any improvement, which may be attributed to the patient's kidney functions being normal. The oral hygiene of CKD patients is poor; hence we as clinicians can improve their oral hygiene by NSPT which in turn would improve the functioning of their kidneys. In future many more longitudinal interventional studies should be done including the assessment of novel urinary biomarkers, to assess the improvement in both the disease outcomes.

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Tables

BASELINE	GI	Control	20	2.31	0.26	< 0.0001**
		Test	20	2.63	0.21	
	PPD	Control	20	7.25	1.29	0.011**
		Test	20	8.35	1.31	
	CAL	Control	20	6.20	1.01	0.202
		Test	20	6.65	1.18	
3MONTHSPOSTOP	Parameters	Groups	N	Mean	SD	P-value
	GI	Control	20	2.00	0.16	0.043**
		Test	20	2.09	0.09	
	PPD	Control	20	5.10	1.21	1.000
		Test	20	5.10	1.07	
	CAL	Control	20	4.55	0.76	0.679
		Test	20	4.45	0.76	

Table 1: Clinical Assessment

*GI- Gingival Index, PPD-Probing pocket depth, CAL- Clinical attachment level

¶N- Sample size, SD-Standard deviation

**P value- Probability value <0.05 statistically significant

Time	Parameters	Groups	N	Mean	SD	P-value
BASELINE	*Treponema denticola*	Control	20	1.53	1.41	0.049**
		Test	20	3.12	2.52	
	Tanerella forsythia	Control	20	1.36	1.27	0.512
		Test	20	1.98	2.05	
3MONTHSPOSTOP	Parameters	Groups	N	Mean	SD	P-value
	Treponema denticola	Control	20	0.78	0.84	0.021**
		Test	20	2.01	1.76	
	Tanerella forsythia	Control	20	0.68	0.82	0.096
		Test	20	1.19	1.11	

Table 2: Microbiological Assessment

*N- Sample size, SD-Standard deviation

**P value- Probability value <0.05 statistically significant

Time	Parameters	Groups	N	Mean	SD	P-Value
BASELINE	Serum creatinine (mg/dl)	Control	20	1.12	0.12	<0.0001**
		Test	20	2.75	0.87	
	Albumin creatinine ratio	Control	20	0.12	0.04	<0.0001**
		Test	20	0.27	0.02	
3MONTHSPOSTOP	Parameters	Groups	N	Mean	SD	P-Value
	Serum creatinine (mg/dl)	Control	20	1.13	0.10	<0.0001**
		Test	20	2.01	0.59	
	Albumin creatinine ratio	Control	20	0.12	0.02	<0.0001**
		Test	20	0.25	0.02	

Table 3: Biochemical Assessment

*N- Sample size, SD-Standard deviation

**P value- Probability value <0.05 statistically significant

Figure Legend

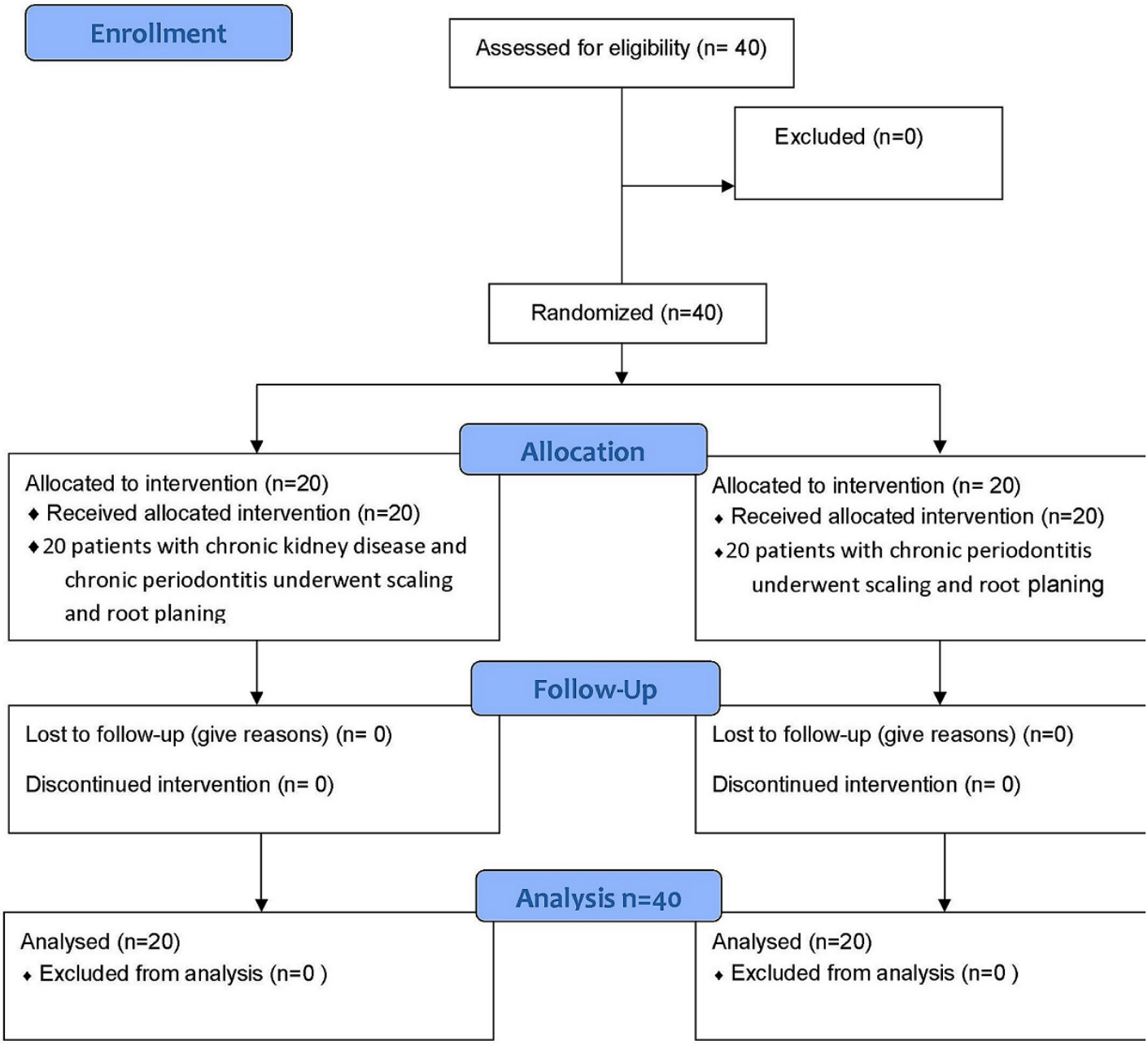

Fig 1 CONSORT Flow Diagram

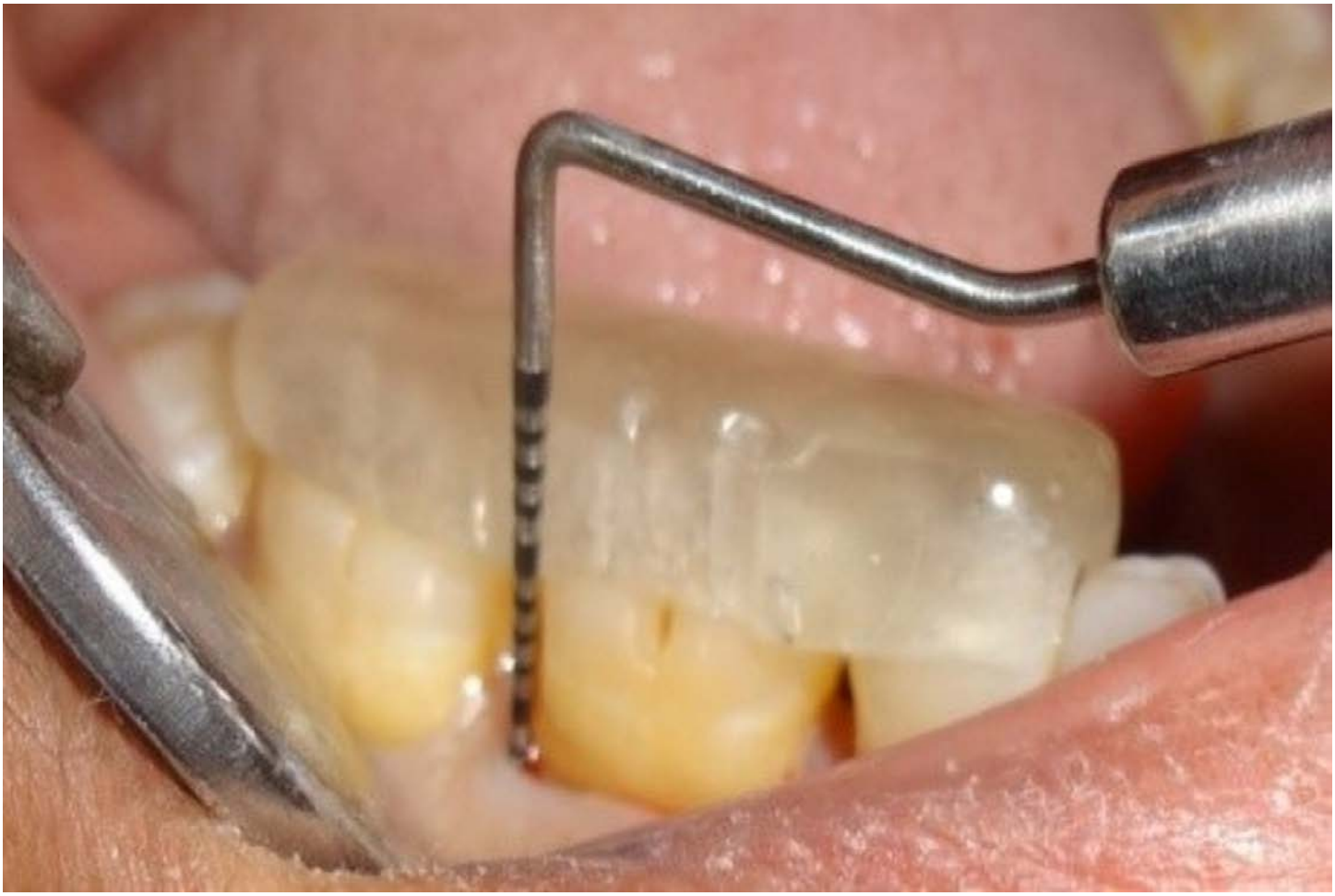

Fig 2 Measuring PPD with UNC 15 Probe

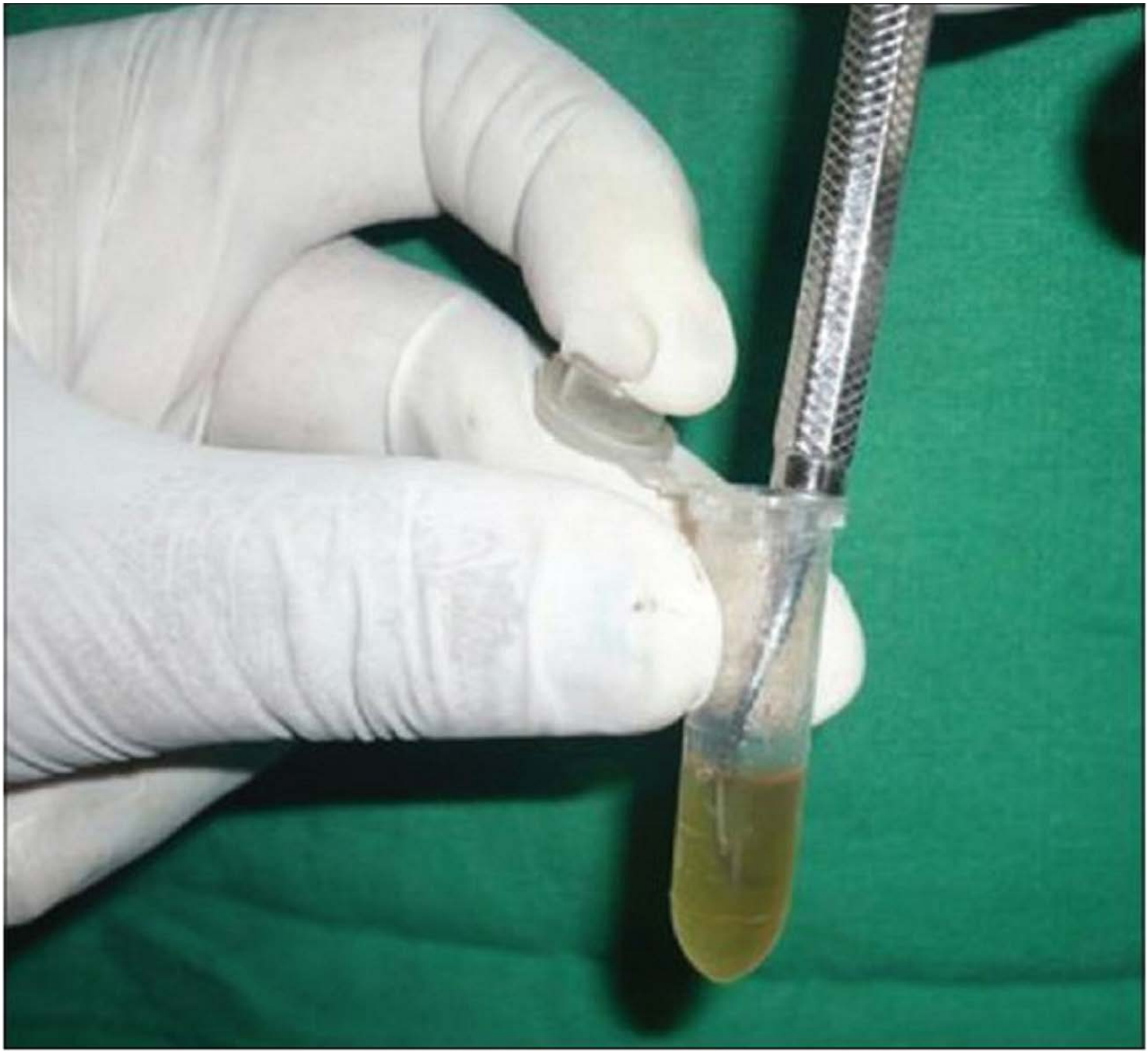

Fig 3 Transferring plaque sample to Eppendorf tube

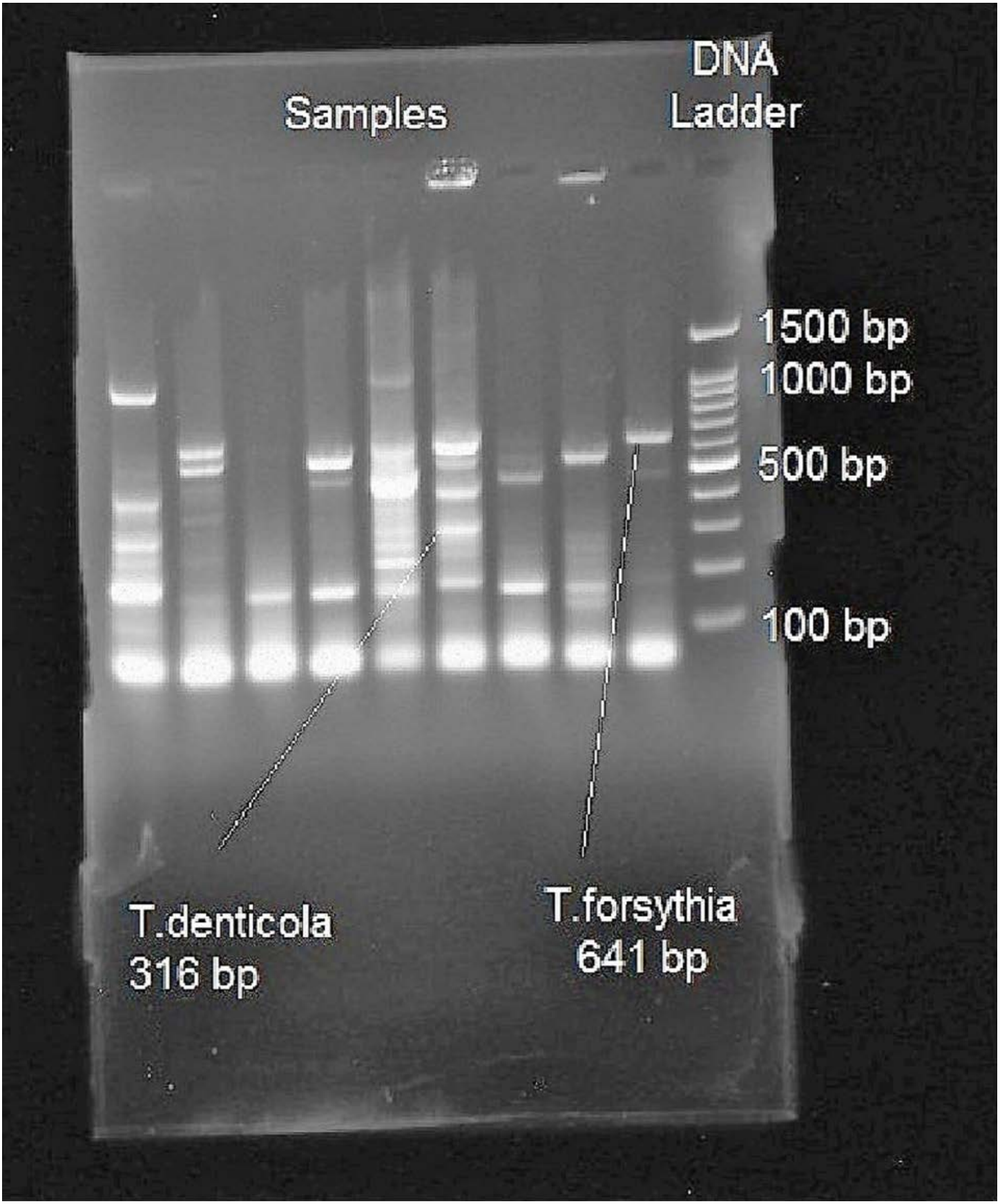

Fig 4 Identification of T. Denticola &T. Forsythia by PCR

Fig 5 Agarose gel Electrophoresis

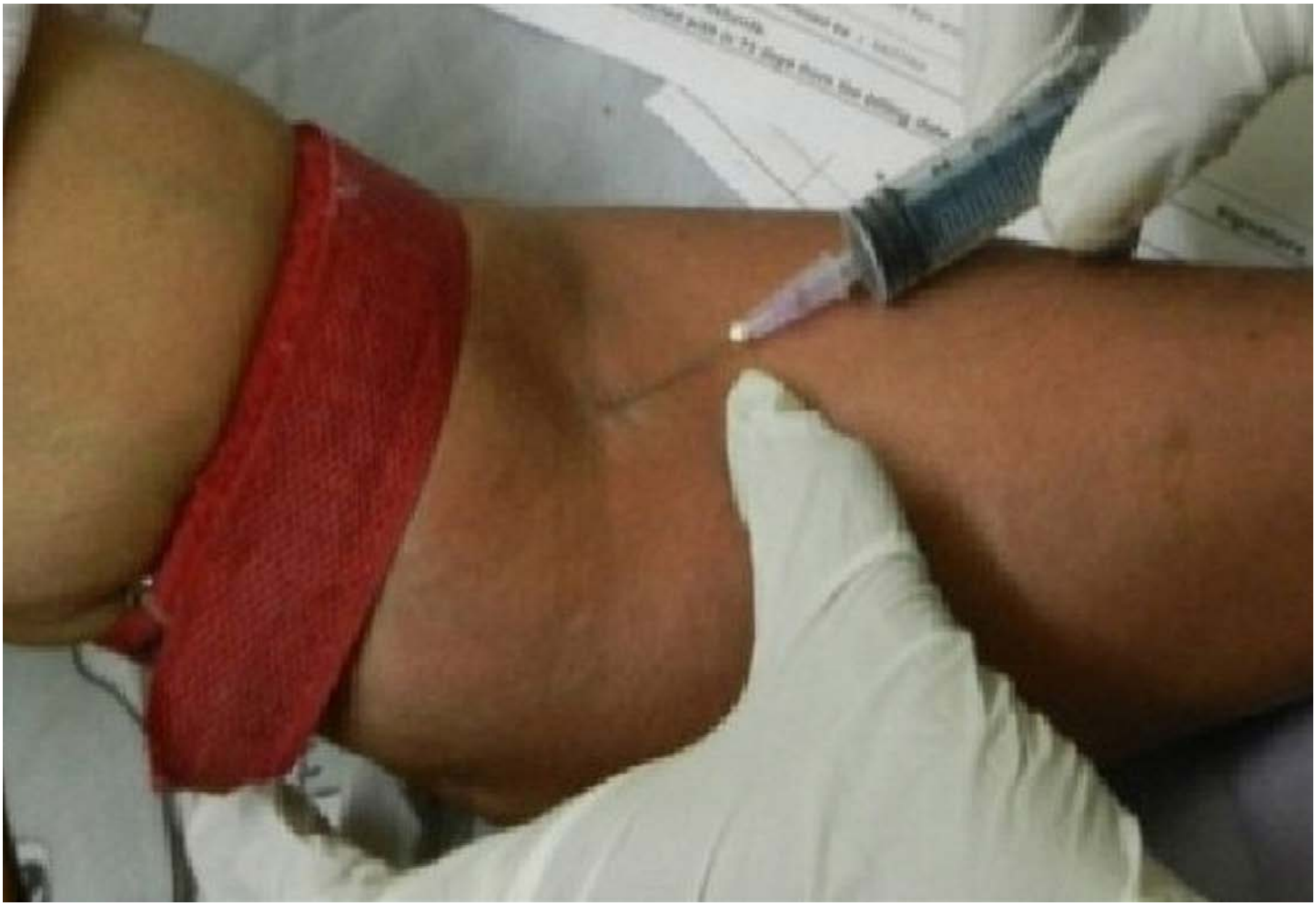

Fig 6 Blood Sample for Serum creatinine.

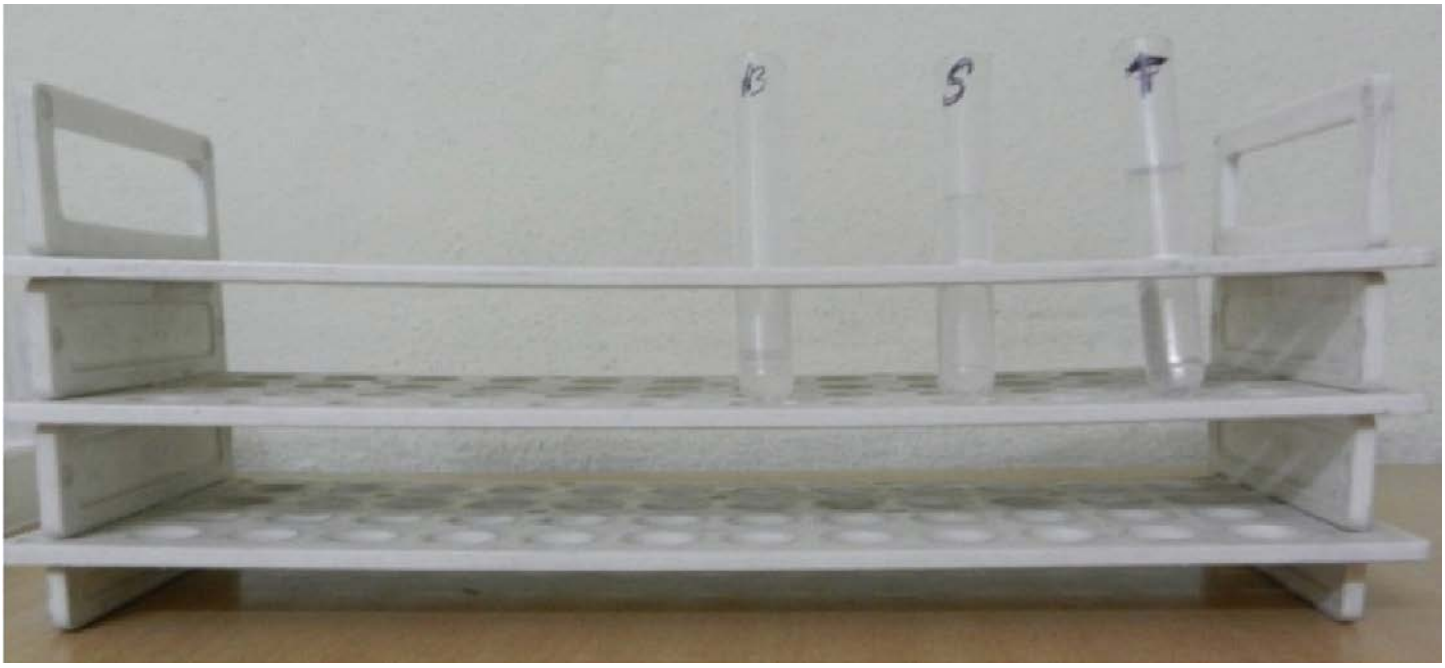

Fig 7 Blank Standard Test for Urinary Albumin: Creatinin