Cytomorphometric Analysis of Exfoliated Buccal Mucosal Cells in Patients with Chronic Kidney Disease

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Abstract

Background: The impact of systemic diseases on oral health is imperative for diagnosis for several diseases. This effect is usually noticed in later stages of the disease or in immunocompromised patients. Patient with chronic kidney disease are generally more susceptible to infection due to decreased immune response. This depression in the general health reflects as changes in the oral mucosa too. Oral mucosal pathological changes can be best assessed through histopathological evaluation. Cytological analysis can also be done to assess the cellular morphological changes. This study will help to analysis the impact of chronic kidney disease on oral health on a cellular level.

Method: This study aims to evaluate the cellular changes induced by chronic kidney disease. Cytomorphometric analysis for 40 subjects – 20 each from healthy and chronic kidney disease group respectively were done by computerised method using Image Pro® 3DS 6.1 software.

Results: Cellular morphological changes like nuclear and cytomorphological changes were assessed and statistically analysed. Results revealed that the mean nuclear and cytoplasmic area in chronic kidney disease group were significantly altered when compared to the control group at $p < 0.05$

Conclusion: Study reveals the cytomorphic changes in oral mucosal cells as result of chronic kidney disease. Thorough research and knowledge about the oral mucosal changes during the entire course of the disease is necessary to evaluate the impact of renal dysfunction on oral mucosal environment and its synergistic effects on the oral health.

Key Words: CKD, Cytomorphometry, Cytology, Oral

Introduction

Oral health of an individual is a primary concern when it comes to maintaining oral hygiene to maintain the equilibrium between aesthetics and functions of the stomatognathic system. We also know that systemic conditions may have an impact on the oral health of individuals. Even though literature gives evidence of several signs and symptoms like periodontally affected teeth, xerostomia, infection prone oral cavity etc in systemic conditions/diseases; Very often this comes into a foray only when oral lesions are taken into consideration for diagnosis in late stages of diseases or in immunocompromised individuals. It is crucial that dentists and dental health care workers are aware of early changes in oral cavity in all systemic disease in correlation with the stage of the disease so as to provide optimal dental care in accordance to the systemic health. This also helps in stabilising the doctor-patient relationship [1, 2].

Several clinical alterations of oral mucosa are known to be seen Chronic Kidney disease (CKD) and in transplant patients. However, there is no other study to evaluate the cellular level changes in early and late stages of the disease (CKD) other than diagnosis of obvious clinical lesions seen in late stages or evaluating changes in transplant patients; which makes the need to evaluate oral cytological changes of oral buccal mucosal cells inured to analysis oral health on a cellular level in patients with renal dysfunction. Kidney ailment is a worldwide disorder with an increased incidence, prevalence and poor outcomes. It also presents with systemic ailments, loss of kidney function, cardiovascular disease and premature death. Diagnosis of acute and chronic kidney failure at an early stage is a major concern. Estimation of GFR is an important part in the assessment of kidney disease [1]. Diagnostic tests based on fluid generally use blood and urine and less frequently the esoteric fluids such as saliva, sweat, and tears. Saliva's popularity has suffered because it lacks "the drama of blood, the sincerity of sweat and the emotional appeal of tears."' Sweat and tears, however, are difficult to obtain in sufficient quantities for routine testing. Saliva, by default, therefore becomes the most favoured alternative to blood [2]. Studies have reported significant high creatinine levels both in serum and saliva of CKD patients compared with controls because the kidneys are unable to excrete creatinine in renal failure and hence its concentration in blood increases. The increased concentration in saliva may be because of high serum creatinine which creates a concentration gradient which in turn causes the diffusion of creatinine from serum to saliva in CKD patients [3-5].

Studies proving such salivary changes in the oral microenvironment predispose us to evaluate the changes in the oral cavity. Oral manifestations of chronic kidney disease include a wide range of oral lesions appearing as white lesions and/or ulcerations [6]. CKD being a progressive disorder may predispose to changes from the early to late stage of the CKD. Cytological analysis to evaluate changes in the oral epithelium will help analyse the changes of oral mucosa in patients with renal dysfunction. The study aims to analyse the cellular changes in the buccal mucosa of patients with chronic kidney disease in comparison with healthy individuals using cytomorphometric analysis software. This will indirectly help to analyse the impact of CKD on oral health of an individual.

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Materials and Methods

Source of samples

- SRM Dental College & General hospital
- Stanley Medical College and Hospital
- Before enrolment, each subject consented to the protocol, which was reviewed and approved by the Institutional Review Board of SRM UNIVERSITY.
- Group I: Healthy Individuals : 20
- Group II: Patients with chronic kidney disease: 20 *(Table 1)*

Inclusion criteria

Patients presenting with symptoms of renal dysfunction

- Patients were classified as chronic kidney disease using MDRD (Modification of Diet in Renal Disease) formula based on the GFR values derived from serum creatinine values.

Exclusion criteria

Pregnancy, Patient's under creatinine supplements.

Methodology

- Smear taken from the subjects – CKD and healthy individuals using a wooden spatula.
- Smears were immediately fixed with a commercial spray fixative containing 95% ethyl alcohol.
- Fixed smears were stained using Papanicalou method.
- Sample blinding was done.
- Prepared slides were analyzed using Image Pro analysis software.

Criteria for Image Analysis

Parameters for analysis

- Nuclear area
- Cytoplasmic area
- Nuclear/Cytoplasmic area

Field

- 40x Magnification
- 5 field of views
- 10 cells per field
- Total of 50 cells per slide.

****Values calibrated in the measure of microns in Image pro 3DS 6.1

Results and Discussion

Oral health is considered to be the mirror of systemic health. Any chronic change in the oral mucosa due to factors other than local causes manifests as changes in the systemic health. Oral manifestations of systemic diseases are a key in the diagnosis and they also denote the influence of systemic health on oral mucosa. Oral manifestations of renal diseases include an array of presentations like altered taste sensation, gingival enlargement, dryness of mouth, parotitis, enamel hypoplasia, delayed eruption, mucosal lesions like oral hairy leukoplakia, lichenoid reactions, ulcerations, angular chelitis, candidiasis, uremic stomatitis, renal osteodystrophy's etc. A very few studies have been done to evaluate the oral mucosal changes in chronic kidney disease based on cytological studies [6]. Direct relationship or the influence of chronic renal diseases on oral mucosa is not clearly understood. Few studies have linked the renal health to the inflammatory state, oxidative stress, DNA damage in salivary gland; this influence on salivary gland structure may correlate to oral findings like altered taste sensation, dryness of mouth, parotitis etc [7-11]. These salivary changes may influence the changes in the oral mucosa and also may have a synergistic effect due to risk factors like smoking, alcoholism, pan chewing etc. Although it has been established that above mentioned habits cause oral epithelial alterations or dysplasia changes, it has not yet been conclusively determined that chronic kidney disease can cause direct alterations to oral mucosa. Additional risk factors should be ruled out in chronic kidney disease before looking into changes caused as a result of chronicity of renal disease. When discussing the changes in oral mucosa due to CKD, systemic factors like diabetes should also be taken into account since many diabetic patients acquire diabetic nephropathy and hypertension as diabetes is a concern and cause for CKD. Regardless of the cause of the disease, CKD is presented with varying levels of kidney damage [12]. Studies pertaining to analysis of the changes in the oral mucosa in chronic kidney disease are very few. Our study aims to analyse the cellular changes in oral mucosa by analyzing the cytomorphometric changes in exfoliated buccal mucosal cells of patients with chronic kidney disease.

Recent advancements in the quantitative techniques and availability of software's for evaluation have made it possible to measure geometric features by using morphometric techniques to evaluate changes in the cellular cytomorphology of oral mucosa.

Cytomorphometric studies have been widely used in exfoliative cytology to analyse cytological parameters like cell diameter, nuclear diameter, nuclear area, cytoplasm area and nuclear-cytoplasmic ratio. Apparent changes in the nuclear area, cytoplasmic area and the nuclear- cytoplasmic ratio have shown to provide a significant data to distinguish between normal healthy mucosa and lesions.

Ogden et al presented that the use of quantitative techniques based on parameters like nuclear area, cytoplasm area and the ratio can enhance the credibility of using software based quantitative study for rapid diagnosis of oral cancer. It is also suggested that the precision and repeatability will improve the sensitivity in the diagnosis [13].

Authors have also attempted to evaluate cytomorphometric changes in systemic diseases like chronic renal failure, and in kidney transplant patients and diabetes [14-16].

Table 1. Demographic Data of control and the study group.

	Group I – Healthy Control	Group II – CKD patients	
Sample size	20	20	Stage I - 1
			Stage II - 7
			Stage III - 4
			Stage IV - 8
No of Males	12	15	
No of Females	8	5	

Table 2. Mean and standard deviation for nuclear area, Cytoplasm area, Nuclear-Cytoplasm area for Group I & II.

Group	Parameters	N	Mean*	Std. Deviation (+/-)
Group I	Nuclear Area	20	53.5483	7.18053
	Cytoplasm Area	20	2758.6277	234.01297
	Nuclear/Cytoplasm Ratio	20	0.0195	0.00302
Group II	Nuclear Area	20	82.1468	7.33251
	Cytoplasm Area	20	2434.622	330.13012
	Nuclear/Cytoplasm Ratio	20	0.0344	0.00592

Table 3. Statistical analysis between each group.

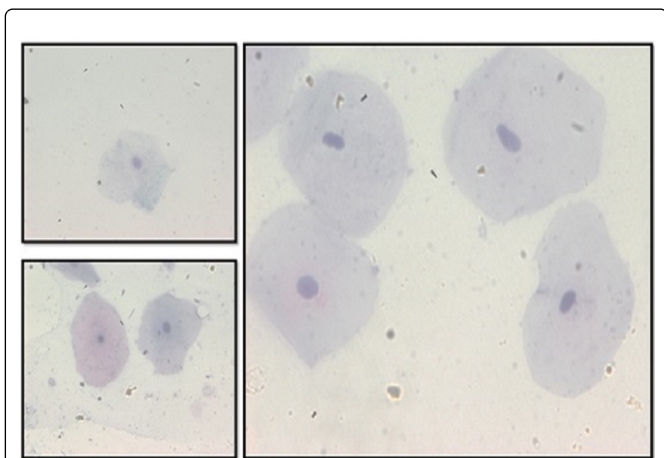| Parameter	Mean and Standard Deviation		T – score	P value	Significance
Nuclear area	Group I	53.5483 ± 7.18053	-12.46211	< .00001	Significant at P < 0.05
	Group II	82.1468 ± 7.33251			
Cytoplasmic area	Group I	2758.6277 ± 234.01297	3.5808	0.000958	Significant at P < 0.05
	Group II	2434 ± 330.13012			
N/C ratio	Group I	0.0195 ± 0.00302	-10.01616	< .00001	Significant at P < 0.05
	Group II	0.344 ± 0.00592			

Figure 1. Cells under 40x magnification taken into criteria for measurement.
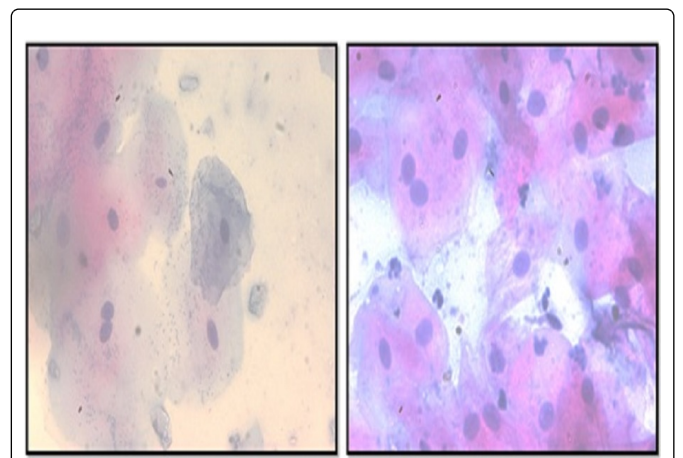

Figure 2. Cells under 40x magnification not taken into criteria for measurement.

In this study, microscopic and cytomorphometric analyses of the oral epithelium in CKD patients were performed. The cytomorphometric findings in the oral smears of CKD patients demonstrated that there were statistically significant differences present in the nuclear area, Cytoplasmic area and Nuclear/Cytoplasmic ratio.

The mean nuclear area in CKD group was found to be greater than the healthy control group. We observed the mean nuclear area in Group I, Group II were found to be 53.5483 ± 7.18053 and 82.1468 ± 7.33251 respectively; *(Table 2)* T-test analysis between these variables revealed a T-score of -12.46211 and p-value < .00001 *(Table 3)*. The observations were found to be statistically significant at p < 0.05. Similarly on analyzing the cytoplasmic area, the mean area Group I, Group II were found to be 2758.6277 ± 234.01297 and 2434 ± 330.13012 respectively; T-test analysis between these variables revealed a T-score of 3.5808 and p-value 0.000958; the observations were also found to be statistically significant at p < .05 . Similarly, the mean measures of cytoplasmic area in Group I and II showed a T-score of 3.5808 and p-value as 0.000958 which were statistically significant at p< 0.05. Hence, we were able conclude statistically significant differences in the mean nuclear and cytoplasmic area between the control and the CKD group (p < 0.05) *(Table 3)*.

Furthermore, analyzing the nuclear/cytoplasmic ratio was also found to be statistically significant on comparing the control (mean: 0.0195 ± 0.00302) and the study group (0.344 ± 0.00592) with a T-score of -10.01616 with p value < 0.001; the results revealed a statistical significance at p < 0.05. Despite significant results, number of other systemic and local factors also needs to be considered before further conclusion.

Routine cytological examination also helped us to observed micro-nuclei and peri-nuclear halo in mucosal cells of patients with late stage of the disease. Increased microbial carriage was also evident in late CKD stages. Microbial carriage in late stage CKD patients could be a reflection of decreased immune response.

Oral mucosa of uremic patients can lead to similar changes along with factors when associated with nutritional deficiencies like iron deficiency anemia and megaloblastic anemia (deficiency of vitamin B12 and folic acid) [17]. Vitamin B12 and folic acid are essential substances for DNA synthesis [18]. These 2 factors, included in nutritional deficiency may impair DNA synthesis and result in changes associated with the nuclear and cytoplasmic size. However, to prove such a correlation, there should be studies related to the vitamin levels and the altered cytomorphology.

Also, the oral cavity is cleaned by saliva which removes food residue from the surface. If the flow of saliva diminishes considerably, the accumulation of bacteria increases in the mouth [19]. It is known that uremic patients have lower salivary flow rates and that their saliva may contain urea [20]. These factors associated with saliva may also predispose to morphological changes in the oral mucosa.

A number of factors that could influence the cytomorphology of oral mucosa cells have been widely investigated in the scientific literature. They include radiotherapy, smoking, alcohol, and malignant oral lesions.

Therefore, the effects of such factors, if present, should be taken into account when assessing a lesion.

Keles et al. suggested that there was a real increase in the nuclear volume in the oral smears of transplant patients, also significant differences in the cytoplasmic volume, and the N/C mean was higher in the transplant patient group compared to the healthy group. They suggested that oral cells may have malignant transformation in transplant patients [15].

Göregen et al. found that the average nuclear area, nuclear perimeter, minimal nuclear diameter, and maximal nuclear diameter values of the buccal mucosa cell nuclei of smokers were higher than those of non-smokers [21].

Our study data has been supplemental to previous studies done to evaluate oral cytology. Exfoliative cytology can be considered for assessment of the mucosal changes in uremic patients. Minimal and evident cellular changes in the oral mucosa in patients with chronic kidney disease predispose to the necessity to evaluate the oral mucosa of CKD patients who may also be exposed to habits like smoking, drinking, tobacco chewing etc. In- depth analysis and evaluation may reveal the underlying pathogenesis and a possible role of renal diseases and other factors that may contribute to the cellular changes in the oral mucosa *(Figure 1,2)*.

Conclusion

Our study revealed the cytomorphometric changes seen in the oral mucosal cells in CKD were statistically significant when compared to healthy individuals. Considering that many other local and systemic factors can contribute to such changes, further analysis is necessary to confirm the oral cytomorphological changes in chronic kidney disease. Literature reveals that also several systemic factors other than CKD may predispose to cellular damage. It is important to take into consideration of all factors that may affect the overall status. It is necessary to evaluate the changes in each stage of the kidney disease. Stage wise analysis considering the role of other confounding parameters will help in analysis of the critical point at which the chronic kidney disease may induce cytological changes in the oral mucosa. This may also give an insight into the stage at which the disease may start producing changes in other organs or environment/mucosa other than the renal system. Evidences derived from our study gives us few insights to verify the impact of renal health on oral health of a patient; it is hence very crucial to follow up with studies that are able to analyze changes with high sensitivity and specificity. It is important to analysis renal health in early stage as well along with other systemic conditions to provide a comprehensive treatment plan. Dentists and oral health care workers can't afford to ignore or misdiagnose even slightest of changes in oral health of a patient with any systemic disease. Growing towards an era of personalized and targeted medications, it will sooner be acknowledged for usage in dental medications as well which further emphasize the need to evaluate oral health in accordance to the general health of an individual.

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