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Salivary Urea and Creatinine as a Diagnostic Marker of Chronic Kidney Disease – Review

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Abstract: *Saliva, a bodily fluid is known to express several proteins on it's own and secretions that diffuse secondarily from the serum. Such an expression is seen in normal physiology and also in pathology. Expression of serum renal function markers – creatinine and urea in saliva has gained greater attention in recent times towards research based on using saliva as a non-invasive diagnostic method. However, there are several isolated studies based on clinical criterias that discuss about the use of saliva as a diagnostic method in patients with chronic kidney disease. This article aims to present an over view of the available literature and discuss about current proceedings and possiblities for future directions.*

Keywords: *CKD, Creatinine, Urea, Saliva, Renal, Urea*

I. Introduction

Saliva, an ultra-filtrate of blood is a human bodily fluid which influences oral health through specific physical and chemical properties. Saliva in normal health is important for lubrication, solubilisation of food, digestion, cleansing of the oral cavity, aids in speech etc. Whole saliva is composed of secretions of the major and the minor salivary glands, mucosal transudations, gingival crevicular fluid, blood derivatives, exfoliated epithelial cells, bronchial secretions, microbial environment, food debris and several other cellular contents. This complex fluid is known to be composed of an array of hormones, proteins, enzyme, antibodies, antimicrobial constituents and cytokines. (1) Studies on whole saliva are known to have been started at least a century ago. The studies range from simple biochemical methods to mass spectrometry, PCR, microarray, nano-scale sensors that have been helpful to establish a list of salivary markers. Saliva is usually preferred over serum for studies because of the non-invasiveness, patient compliance, and sample feasibility etc(2–6)

Studying saliva from a diagnostic point of view has been the area of research in recent times due to easy sampling options available with saliva. Using saliva as a study medium is cost-effective, non-invasive and accurate. The option of detecting pathology at an early stage will effect patient comfort, prognosis, therapeutic modalities, monitoring the disease, survival rate and recurrence. Saliva, being a part of the oral microenvironment has several known established biomarkers in health and in pathological conditions. Studies have enabled us to discover that saliva based microbial, immunologic and molecular markers will help evaluate the condition of both healthy and the diseased individuals. Salivary biomarkers are also known to be expressed in oral premalignant and malignant conditions. Study of salivary biomarkers as a diagnostic medium in systemic health conditions has been of recent interest. Saliva is known to be used as an investigational aid in the diagnosis of systemic conditions that affect the salivary glands and the composition of saliva – as seen in Sjogren's syndrome, alcoholic cirrhosis, cystic fibrosis, sarcoidosis, diabetes mellitus and diseases of adrenal cortex.(7) There are saliva based studies that have analysed renal function markers – creatinine and urea in chronic renal diseases, patients under dialysis and transplant. Our review aims to discuss the use of salivary creatinine and urea in the diagnosis; also to understand pathobiology of expression of these serum markers in saliva in chronic renal disease.

II. Pathobiology Of Serum Markers In Saliva

2.1 Mechanism of expression of serum markers in saliva in normal health:

Saliva a bodily fluid known to contain molecules as seen in blood. It is a clear bio-fluid composed of 99% water, proteins 0.3% and remaining as inorganic components. (7) Saliva is considered to be an alternative to serum for the following reasons: non-invasive, easy sampling, feasibility of multiple sampling, collection

technique that is easy and painless, safer to handle, less infectious, easy storage and transport, and good patient compliance etc. Expression of serum markers in saliva in normal conditions, alterations in oral health and systemic conditions have enabled studies to determine the use of saliva as an alternative medium to serum for diagnostic purpose. Salivary glands locally produce most of the organic compounds. Some molecules expressed in saliva are due to transfer of molecules from the blood. Intra and extra cellular transport mechanisms are thought to be reasons for diffusion (active or passive) of molecules from blood into saliva. (8) Saliva earlier considered only as digestive juice is now seen as a potential diagnostic medium due to expression of the serum markers.

Salivary glands are highly permeable and are surrounded by capillaries. This environment will facilitate the free exchange of blood based molecules into the salivary gland acini. This transport of molecules is due to either transcellular (Passive and active transport) or paracellular (extra cellular ultrafiltration) mechanisms.(7) Diffusion of molecules is considered to be the common route for molecules to transport from blood to saliva. The ability of molecules to diffuse depends on the size and the electric charge that is carried by the molecules. Active transport of molecules into the saliva through secretory cells of the salivary glands is another mode of transport. Ultra-filtration is an extra cellular mechanism for transport of blood substances into saliva by filtration through the spaces between the acinus and the ductal cells. Only very small sized molecules can be transported through ultra-filtration. It may also filter through the gap junctions between cells of secretory units. These smaller sized molecules transferred through ultra-filtration is usually found into minimal (300 to 3000 times) concentration in comparison to their plasma levels. Blood molecules may enter oral cavity through gingival crevicular fluid which is produced in the gingival sulcus. (8) These are the known mechanisms for transport of blood components into the saliva.

2.2 Salivary urea in renal pathology:

Forland et al (1964), Dahlberg et al (1977) and Shanon et al (1977) have demonstrated the proportional levels of parotid salivary urea level to that of blood urea level of patients under hemodialysis in their earlier studies. Later in 1987 Kyaw Tun Sein and Geetha Arumainayagam were the among the first few authors to demonstrate urea levels in mixed saliva and found a correlation between the blood urea levels in normal healthy individuals, and patients with diabetes, hypertension or chronic renal failure.(9)Their observation were similar to the results of Akai et al who developed a salivary strip test method and an automatic reflectance spectrometer to analyse salivary and blood urea levels and found a significant correlation in blood and saliva levels. (10) Khramov et al reported that salivary urea could reflect the progression of renal dysfunction. Their study showed expression of salivary urea and ammonia correlated with the respective blood levels in patients with kidney disease and healthy individual. It was also noted that treatment for the disease resulted in the changes in the salivary & blood levels of urea and ammonia. (11) Estela et al in 2009 determined the urea levels in normal and chronic renal failure individuals and found a positive correlation. They had reported a study population consisting of patients with hypertension (30 %), diabetes mellitus (15 %), renal polycystosis (12 %), interstitial nephritis(8 %), renal stones (7 %), chronic pyelonephritis (5 %), miscellaneous and unknown as the etiology for chronic renal failure. The study revealed 100% specificity and sensitivity for optimised cut off levels of 7.5 mmol/L &8.20 mmol/L for Salivary urea and blood urea levels. Healthy subjects in their study reported a mean salivary urea level of 5.36 mmol/L. It was suggested that the uremic status of individuals can be identified with salivary urea levels considering that their study showed a 100% percent sensitivity and specificity at an optimised cut-off level. (12) Maria et al demonstrated in a study to validate the use of salivary urea for diagnosis of chronic kidney disease. Subjects were enrolled in this study based on the creatinine clearance instead of GFR since the latter can be less accurate in certain populations. This study also demonstrated an increase in salivary urea concentration in chronic kidney disease when compared to the normal healthy volunteers. ROC (Receiver Operator Curve) analysis in their study revealed a cut-off point at 20mg/dL at high sensitivity and low specificity, an optimised cut off level was determined to be 40 mg/dL in this study. (13) Xia et al also tried to see the changes of salivary urea, creatinine and uric acid in healthy and chronic kidney disease patients. The study showed a correlation between the serum and salivary urea levels in healthy and CKD individuals. It was noted that the salivary urea levels were higher in the late CKD stages compared to the early stage. ROC curve analysis for salivary urea in this study was 0.898. (14) Peng et al studied the influence of age, gender and collecting time among chronic disease group and the normal group. Their study revealed an increase in the concentration of urea in saliva of diseased group compared to the normal group. The study concluded that the concentration of salivary urea were not affected by the collecting time, age and gender but it was related to the age and levels of blood urea. . This study revealed high correlation between salivary urea and blood urea levels in patients (r=0.980) and normal subjects (r=0.907). Syed Parveez Ali et al demonstrated the levels of salivary and blood urea levels in end stage renal failure patients which included 21 patients from hemodialysis group and 15 patients from normal group. Their study showed no statistical significance between the blood urea and salivary urea in the hemodialysis group. However, mean levels of blood and salivary urea between hemodialysis

and control group revealed a statistical significance. (15) Reda Sedkey 2014, showed a positive correlation between serum urea and saliva urea in the healthy group, CKD patients, and ESRD patients.(16)Taye Jemilat 2016 also showed positive correlation with the salivary and plasma levels in urea. Cut off value was determined as 27.50mg/dL at 0.86 sensitivity 0.07 specificity.(17) Shruthi et al in 2016 estimated the salivary urea levels in various stages of chronic kidney disease (CKD). Significance was seen in the salivary urea mean levels between the control and the chronic kidney disease. Significance was also seen within the stages of the CKD. However, this study did not correlate the salivary and serum urea levels but went on to show the significance between and within the groups which further validated the significance of use of salivary urea as a diagnostic medium.(18) Several studies conducted over the years to demonstrate the use of salivary urea as a diagnostic medium have contributed to the knowledge pertaining to the use of salivary urea as a diagnostic medium. A standardised cut off value for different stages of CKD have not been established even though studies have shown cut-off values determined at varied sensitivity and specificity. It is however established that salivary levels of urea correlate to the serum levels which contributes the notion of using saliva as diagnostic medium.

2.3 Salivary creatinine in renal pathology:

Serum creatinine is considered to be a gold standard renal function test measure along with serum urea to determine the renal function.(19) Jonathon Lloyd et al in 1996 was among first few authors to analyse salivary creatinine levels in patients with renal diseases. Earlier studies revealed that salivary creatinine was about 10-15% of the serum levels, this expression of serum markers in saliva was considered to be due to the ultrafiltration of creatinine into saliva. Their study revealed a significant correlation in the renal disease group (r=0.784) but did not show good correlation in healthy individuals (r= 0.259). Cut off value was 16.8 mmol/L at 100% sensitivity and 20.6 mmol/L at 100% specificity.(20) Xia et al in 2012 demonstrated the levels of urea, creatinine and ureic acid in chronic kidney disease group. Levels of salivary creatinine in the CKD patients were significantly higher than those of healthy people. Salivary creatinine concentrations of middle and late stage CKD patients were obviously higher than those of healthy people and early stage CKD patients ($P<0.05$). The correlation between the serum and salivary urea levels in healthy and CKD individuals($r=0.932$ and $r = 0.971$). Xia also demonstrated area under the curve (AUC) of the ROC of Urea in of CKD to be 0.898 which was found to be statistically significant. Sensitivity and specificity was 0.806 and 0.968 respectively.(14) Reda et al showed that there was significant positive correlation ($P < 0.05$) between serum creatinine and saliva creatinine in the healthy group, CKD patients, and ESRD patients ($r = 0.83$, 0.58, and 0.89, respectively).(16)

Venkathapathy et al revealed correlation between the salivary and serum levels of creatinine in the chronic kidney disease group (r=0.731), found to be significant at p value < 0.05. The study showed a negative correlation between the serum and salivary creatinine in the control group ($r = -0.326$) with significance at $p < 0.05$. Study revealed that control group had a mean serum creatinine of 0.89mg/dL (SD 0.168) and the salivary values with a mean of 0.12mg/dL (SD 0.06). In CKD patients, the serum creatinine level had a mean of 5.96mg/dL (SD 3.048) and salivary creatinine level was found to be with a mean of 0.66mg/dL (SD 0.485). Comparison of serum and salivary creatinine levels between CKD patients and controls was found to be significant at $p <0.001$. The study demonstrated the total area under the curve obtained as 1.000 for serum creatinine and 0.967 for salivary creatinine. Sensitivity and specificity for different values of salivary creatinine were established and a cut-off value of 0.2 mg/dL was determined as this gave a best trade-off with sensitivity of 97.14% and specificity of 86.5%. (21) Compared to salivary urea, there are fewer studies that have analysed salivary creatinine in renal diseases. Few studies have determined cut off values but a standardised value is still not established for salivary creatinine that could replace serum as a diagnostic medium.

Currently no study has determined for salivary urea and creatinine levels in comparison to their serum levels in a stage (I, II, III, IV& V) wise manner of CKD. Oral health status and factors that influence the salivary creatinine and urea levels should be reviewed before considering saliva as a diagnostic medium in renal patients. Also, studying the implications of salivary creatinine and urea on general oral health, potentially malignant oral disorders and oral cancer should be done to understand the role of renal function status on oral microenvironment.

III. Discussion

Saliva is considered as a non-invasive diagnostic medium as a result of studies that have shown the expression of serum biomarkers in disease. As said earlier, the biomarkers can be seen in saliva due to diffusion (active or passive) or as a result of ultra-filtration. It is a default observation that when a molecule's concentration increases in blood there can be a diffusion of these molecules into the saliva and thereby causing increase in concentration of the markers in saliva as well.(7) Similarly, serum creatinine and blood urea which are considered as a gold standard for renal function are seen expressed to be in saliva with their gradual increase in serum. Measuring GFR with the creatinine values is an indirect measure of the renal function.(22) Saliva, which also expresses these molecules due to a concentration gradient diffusion may also be used a measure of

renal function if the values are standardised and risk factors are also taken into consideration. Our review will discuss about i) systemic and local factors that influence the expression of salivary markers; ii) influence of systemic factors on local factors and vice versa. Primary risk factors of serum expression to be considered are diabetes, and hypertension as they are the common causes for deterioration of renal health and causing kidney diseases.(22) These systemic factors causing CKD will also cause the expression of markers in saliva. Secondary risk factors include changes in the oral health that may also affect the expression of these salivary markers. Oral cavity which is considered as the mirror of the systemic health is a reflection of the impact of systemic changes on oral micro-environment. Alteration in the systemic environment may affect the oral mucosa, salivary gland function resulting in salivary changes like alteration in the composition & alteration in pH which may affect the caries status of an individual; and periodontal health status.(23)

Diabetes mellitus is an etiological factor for CKD. Around one third of type I diabetes develop into nephropathy in about 16 years; in the same time period one fifth of type II diabetes develop into nephropathy. Incidence is difficult to assess in early stages as 30-50% of patients remain undiagnosed for 5 – 10 years. Progression of diabetic nephropathy to end stage renal disease depends on the control of blood glucose and blood pressure levels.(22) Studies regarding the estimation of salivary glucose in diabetes individuals have discussed the cause of expression of salivary glucose as diabetic membranopathy which results in the leak of molecules across the basement membrane and raised transport of glucose from blood to saliva. Diabetic membranopathy in patients with diabetic nephropathy may be one of the reasons for expression of markers like creatinine and urea to be also expression in saliva apart from the usual diffusion due to concentration gradient.(24)

Hypertension is known to be a cause and consequence of CKD.(22) Arterial wall thickening in hypertensive patients is one of the reasons for decreased flow of blood for filtration in the kidney and thereby resulting in less filtration of molecules that will cause an increased concentration of unfiltered molecules in blood. (25) Increased concentration of molecules in blood may perfuse through the vasculature surrounding the salivary glands and enter saliva as a result of diffusion.

Increased prevalence and death rate due to cardiovascular disease (CVD) in Indian population have overshadowed the occurrence of diabetic nephropathy. Evidence also suggests that CVD is an independent risk factor for CKD.(22) Direct relationship between CVD and reasons for salivary expression of molecules is not well understood. However, a relationship between diabetic and hypertensive state of individuals and salivary markers can be established as diabetes is a common cause of CKD, whereas hypertension is a known cause and consequence of CKD.

The effect of renal diseases with or without associated diabetes and hypertension on salivary gland functioning and structural changes contributing to expression of renal function markers needs to be well understood. Salivary gland is closely associated with the oral cavity. There are several oral manifestations of renal diseases 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 osteodystrophies etc.(23) Changes like altered taste sensation, dryness of mouth, parotitis are directly related to pathological or functional changes pertaining to the salivary gland. A direct or indirect relationship between the progression of renal disease and alteration in salivary gland function is needed. Clarra Ersson et al revealed that CKD patients who are not on dialysis had a significantly more DNA strand breaks in salivary tissue compared with the healthy individuals. Inflammatory markers were also measured and were found to be higher in CKD patients than healthy individuals. They are also exposed to oxidative stress which has been suggested as a reason for high morbidity and mortality rate in the patient group. Oxidative state is related to the expression of inflammatory markers which again is a risk factor for CKD patients. Previous studies show increased levels of DNA damage in peripheral blood mononuclear cells (PBMCs) of CKD patients. This suggests that even peripheral blood cells are affected in CKD. The study suggests that dialysis patients had fewer DNA strand breaks in minor accessory salivary glands than controls, suggesting that peripheral tissue is differently affected by CKD than leukocytes (26). Bibi et al revealed that salivary oxidative stress markers like Salivary peroxidase and Superoxide dismutase were found to be in higher concentration - 15% and 35% respectively than their serum levels.(27) These studies have demonstrated damage mainly in the end stage renal disease and dialysis group. All these factors suggest that there could be salivary gland tissue damage with the progression of the chronic kidney disease. The progression of the disease may be reflected with the salivary gland tissue damage due to above mentioned factors like oxidative stress, inflammation, DNA strand breaks etc. It is also suggested smoking habit may induce salivary gland damage due to nicotine induced genotoxic effect. Similarly DNA damage may be mediated by production of reactive oxygen species due to high serum glucose levels, AGEs (Advanced Glycation End products), free fatty acids and insulin in diabetic individuals. (28) Knas et al evaluated the oxidative damage caused to salivary glands due to streptozotocin induced diabetes mellitus and concluded that diabetes induced oxidative stress is a process taking place in salivary glands, which is independent of the general oxidative stress of the individuals and parotid

glands were more susceptible to oxidative damage. Diabetes induced damage should be considered since it is an important risk factor in CKD.(29) Similarly there may be a possibility that oxidative stress may be produced in salivary glands with progression of renal disease which may be independent of the general oxidative stress. Clinically since it is noted that symptoms are expressed when there is reduction in GFR by 50% which correlates to stage III CKD, we suggest that this stage might be the beginning of peripheral tissue damage (including salivary gland) that may result in altered or excessive expression of markers with progression of disease. We propose an hypothesized model (Chart 1) for salivary expression of molecules adapted from a model given by Prathiba et al based on mechanisms involved with chronic kidney disease – diabetes, hypertension and cardiovascular disease(30).

IV. Chart

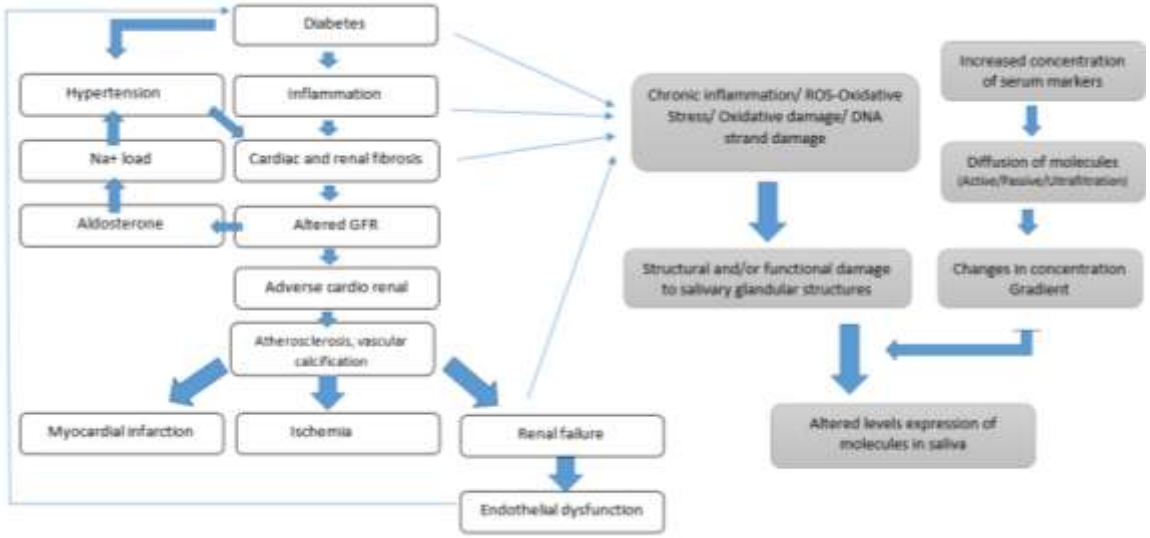

Chart 1: Systemic influence on salivary structures and secretions

V. Future Directions In Renal Pathology

Diagnostic use of salivary renal function markers like creatinine and urea have been much discussed and studied over the years. However, as a biomarker, studies should validate the expression of these markers right from early to late stage of the disease. Most of the studies done have population from late stages of disease, renal failure, haemodialysis or transplant individuals. It is evident from studies that salivary expression will be seen in late stages of the disease; however, to validate a marker or a diagnostic medium, it is necessary that expression is seen in all stages of the disease. In CKD, it is known that clinical symptoms are expressed only when there is 50% reduction in GFR which will correspond to stage III CKD; hence it becomes a challenge to analyse the disease even at early stage (stage I and II).(31) Hence, to diagnose the disease with saliva becomes even more difficult with altered concentration of molecules in saliva compared to blood levels without standardised salivary values and sensitive analytic methods. It is known that expression of markers in late stages of the disease is evident with the advanced nature of the disease. Expression of salivary markers needs to be standardised in early and late stages if it has to be used as an early diagnostic medium, but it will be still viable if used as a marker for monitoring disease as non-invasive, home based monitoring for patients under dialysis, transplant, elderly individuals etc. Salivary strip monitoring could be used by general practitioners and dentists for chair side screening of patients. Serum Cystatin C is seen as a sensitive renal function marker in recent studies to estimate the GFR.(32) Estimation of Salivary cystatin-c will add more input to use saliva as a diagnostic medium for renal disease as cystatin-c is a sensitive marker. However, expression of cystatin-c in saliva and gingival crevicular fluid is seen in periodontal diseases.(33,34) A well designed study to evaluate salivary cystatin-c in renal diseases will reflect a better result in using saliva a diagnostic medium. Salivary strips and lab-on-chips could play a major role in salivary diagnostics in the future of renal diseases.

VI. Conclusion

Standardization of salivary values in all stages of renal diseases following well designed studies involving oral risk factors and standardized techniques in analysis will pave way for the use of saliva as a diagnostic medium and monitoring of renal diseases.

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