**Original Article**

# No Relationship between Serum and Salivary $\beta_2$-Microglobulin Levels in A Sample of Adult Diabetic Men with Chronic Kidney Disease without Renal Replacement Therapy

**Ahmadreza Assareh, M.D.[1], Habib Haybar, M.D.[1]*, Hosein Malekzadeh, D.D.S.[2], Leila Yazdanpanah, M.D.[3], Mohammadreza Bozorgmanesh, M.D.[4]**

**1. Cardiovascular Research Center, Golestan Hospital , Ahvaz Jundishapur University of Medical Science, Ahvaz, Iran**
**2. Department of Oral Medicine, Ahvaz Jundishapur University of Medical Science, Ahvaz, Iran**
**3. Diabetes Research Center, Ahvaz Jundishapur University of Medical Science, Ahvaz, Iran**
**4. Prevention of Metabolic Disorders Research Center, Research Institute for Endocrine Sciences (RIES), Shahid Beheshti University of Medical Sciences, Tehran, Iran**

***Corresponding Addresses: P.O. Box: 173, Cardiovascular Research Center, Golestan Hospital, Ahvaz Jundishapur University of Medical Science, Ahvaz, Iran***
***Email: dr.habibhaybar@yahoo.com***

**Received: 11/Nov/2012, Accepted: 6/Jul/2013**

Abstract

**Objective:** $B_2$-microglobulin ($\beta_2$M) associated amyloidosis is an inevitable complication of chronic kidney disease (CKD). Testing $\beta_2$M in the blood is invasive and expensive. On the other hand, oral fluid is a perfect medium to be explored for public health and disease surveillance. However, it has never been studied if salivary concentration of $\beta_2$M reflects its concentration in the serum. The current study; therefore, aimed to examine the relationship between salivary and serum $\beta_2$M in a sample of adult diabetic men with CKD.

**Materials and Methods:** Among diabetic patients referred to the Nephrology Department of The Golestan Hospital of Ahvaz due to CKD, 40 men not requiring renal replacement therapy were consecutively recruited for this cross-sectional study. Patients were excluded if they had any disease or were using any drugs that might affect the oral mucosa or saliva. The concentration of $\beta_2$M was measured in both serum and saliva. The correlation between serum and salivary $\beta_2$M was measured by calculating spearman's ρ.

**Results:** The Spearman's ρ for correlation between serum and salivary $\beta_2$M was -0.017 (p=0.917), indicating lack of correlation. Serum and salivary creatinine (Spearman's ρ=0.54; p value<0.001) as well as serum and salivary urea nitrogen levels (Spearman's ρ=0.39; p value=0.014) were correlated.

**Conclusion:** Salivary $\beta_2$M levels poorly agreed with serum $\beta_2$M levels, and thus may not be used as a surrogate for serum $\beta_2$M in CKD patients who did not require replacement therapy.

***Keywords:*** $\beta_2$-Microglobulin, Chronic Kidney Disease, Saliva

Cell Journal(Yakhteh), Vol 16, No 2, Summer 2014, Pages: 179-186

**Citation: Assareh AR, Haybar H, Malekzadeh H, Yazdanpanah L, Bozorgmanesh M. No relationship between serum and salivary $\beta_2$-microglobulin levels in a sample of adult diabetic men with chronic kidney disease without renal replacement therapy. Cell J. 2014; 16(2): 179-186.**

## Introduction

The number of patients with chronic kidney disease (CKD) is rising rapidly worldwide (1), and CKD is increasingly recognized as a global public health burden (2, 3). Diabetes mellitus is the most common cause of end stage renal disease (ESRD) in many countries, and it has been estimated that 366 million people will have diabetes mellitus by 2030 (4). Furthermore, diabetes and CKD exhibit synergistic associations with cardiovascular disease and premature mortality (5). There is now a plethora of evidence to indicate that CKD can be detected using simple laboratory tests, and that timely treatment can prevent or delay complications of decreased kidney function, slow the progression of kidney disease, and reduce the risk of

cardiovascular disease (CVD). These advances must be translated to simple and applicable public health measures. Developing a public health policy to improve outcomes entails the understanding the relationship between CKD and other chronic diseases (2).

Kidney failure requiring renal replacement therapy (dialysis or renal transplantation) is the most visible outcome of CKD. However, CVD frequently complicates CKD and individuals with CKD are more likely to die of CVD than to develop kidney failure (6-10), this disease could potentially be treated and prevented among patients with CKD (11, 12).

$B_2$-microglobulin ($\beta_2$M) associated amyloidosis is considered an inevitable complication of chronic hemodialysis (13). $\beta_2$M constitutes a light chain of the class I major histocompatibility complex. Widely distributed in nucleated cells in the body, $\beta_2$M is especially rich in immunocompetent cells, such as lymphocytes or monocytes. Various stimuli cause substantial amounts of the molecule to be shed into the circulation (14). Circulating $\beta_2$M is filtered through the glomeruli and is reabsorbed and metabolized in the proximal tubules of the kidneys (14). Therefore, the plasma concentration of $\beta_2$M is largely affected by the glomerular filtration rate (GFR) of the kidneys. $B_2$M has recently been shown to be related to risk factors of the atherosclerosis, coronary heart disease (15, 21), cardiovascular (20) and total mortality (15, 21).

Due to peripheral venous access difficulty in CKD patients, the plasma concentration of $\beta_2$M microglobulin is not applicable to them. Saliva, as a unique fluid of diagnostic medium, has advanced exponentially in the last decade. While testing $\beta_2$M in the blood is invasive, there are less invasive methods available to test $\beta_2$M in saliva. The ability to measure and to monitor a wide range of molecular components in saliva and to compare their levels to plasma components levels have made possible to study microbes, chemicals, and immunologic markers (22, 23).

It has, however, not been studied if salivary concentration of $\beta_2$M reflects its concentration in the serum. If it has been the case, salivary $\beta_2$M would have provided a unique opportunity as a simple chair-side tool for periodical assessment of patients with CKD. The current study, therefore, aimed to examine the relationship between salivary and serum $\beta_2$M in a sample of adult diabetic men with CKD.

## Materials and Methods

### *Patients and design*

Among diabetic patients referred for CKD to the Nephrology Department of the Golestan Hospital of Ahvaz, Ahvaz, Iran, 40 male were consecutively recruited for the current cross-sectional study. Patients were excluded if they had parageusia (4), were smoker (2) or using any drugs having affected their salivary flow or content, or if there was any other evidence of a systemic disease affecting the oral mucosa or saliva. Patients who required renal replacement therapy were also excluded. Renal replacement therapy is initiated once patients have stage 5 disease or signs of uremia, including lack of appetite, nausea, vomiting, acidosis, hyperkalemia, or fluid overload (24).

### *Measurements*

After a period of 8 to 12-hour overnight fasting, non-stimulated saliva samples were taken from all participants. Participants were instructed not to speak during the saliva collection period. To prevent changes in salivary composition during a 24-hour period, they were instructed not to eat, drink, and use toothbrush, toothpaste, or mouthwash since 2 hours before sample collection. Participants were asked to spit their saliva into a test tube 5 minutes after they washed and rinsed their mouth with water. The sampling was then continued until 10 ml of saliva specimen was collected. A Blood sample (5 ml) was also taken from all participants via venipuncture. Serum and salivary $\beta_2$M was measured using Minineph Human Kit (Binding Site Co., UK), while urea and creatinine in saliva and blood samples were measured using Pars Azmun Kit (Pars Azmoon Inc., Tehran, Iran).

### *Definitions of terms*

GFR was estimated using Cockcroft-Gault equation (25):

$$C_{Cr} = \frac{(140 - age) * Weight * GF}{P_{Cr} * 72}$$

The gender correction factor (GF) is 1.00 for

men and 0.85 for women. We did not used the equation derived from the Modification of Diet in Renal Disease (MDRD) study because it was not validated for diabetic kidney disease (2).

CKD was defined as kidney damage for ≥3 months and/or GFR <60 ml.minute$^{-1}$ per 1.73 m$^2$ for ≥3 months with or without kidney damage. Kidney damage was defined as structural or functional abnormalities of the kidney, initially without decreased GFR (26). ESRD was ascertained in participants with GFR <15 ml.minute$^{-1}$ (27).

Xerostomia was defined clinically. According to the medical literature, dryness of the cheek mucosa was determined by visual examination, palpation and adherence degree of mucosal surface using a wooden spatula (28). Hypertension, diabetes, smoking, and parageusia were diagnosed by self-reporting.

### *Statistical analysis*

Data are presented as either mean (SD) or frequency (%) for continuously-and categorically-distributed variables, respectively. Median [interquartile range (IQR)] used for continuously distributed variable revealed that distribution was not normal.

The linear regression model was used to examine the statistical significance of the association between serum $\beta_2$M, while controlling for age. We used Spearman's ρ as a coefficient of concordance between serum $\beta_2$M and salivary $\beta_2$M, of concordance between serum urea nitrogen and salivary urea nitrogen, and of concordance between serum creatinine and salivary creatinine.

Instead of using arbitrary predetermined cut-points to capture nonlinear aspects of association, we used restricted cubic splines functions of the salivary $\beta_2$M to represent their continuous relationship with the serum $\beta_2$M, so that the relationships were meaningfully in accordance with substantive background knowledge. Splines functions, as phrased by Harrell, are "piecewise polynomials within the intervals of a variable that are connected across different intervals of that variable" (29). Restricted cubic splines function enabled us to use flexibly model continuous predictors (salivary $\beta_2$M), while allowing us to control over the excessive instability and tendency of spline functions in order to generate artifactual and uninterpretable features of a curve. Multivariate restricted cubic splines were used with 4 knots defined at 5$^{th}$, 25$^{th}$, 75$^{th}$, and 95$^{th}$ percentiles (29). In variable selection, we dropped a variable if its removal caused a non-significant increase in deviance.

We used several criteria to compare the overall predictive values of alternative models. In addition, the outcome variables are referred to goodness-of-fit when a model effectively describes them. We used following measures of goodness-of-fit.

1. *Deviance* compared the fit of the saturated model to the fitted model. This was a small value if the model was good. For purposes of assessing the significance of non-linear terms, the values of D with and without the non-linear terms were compared by likelihood ratio test.

2. *Akaike information criterion* (AIC) was used to account for complexity. Difference in AIC >10 was considered significant (30).

### *Ancillary analysis*

In order to apply ancillary analysis, we examined the predictive ability of the serum and salivary $\beta_2$M for renal failure. Owing to high prevalence rate of renal failure in the study sample, the odds ratios obtained from the logistic regression model would be excessively large. When a study outcome is rare, the odds ratio estimate of causal effects will approximately be the risk ratio. However, if a study outcome is common (>10%), the odds ratio will be further from 1 than the risk ratio (31, 32). We, thus, used the Cox proportional hazard regression model with time as constant variable to approximate risk ratio in order to avoid the renal failure for increasing levels of $\beta_2$M.

The significance levels for selection of spline functions by backward elimination were set at 0.1. For salivary $\beta_2$M, however, we set the significance level at unity, forcing it into the model, leaving the others to be selected or not. For the rest of the analyses, we set the statistical significance level at a two-tailed type I error of 0.05. All statistical analyses were performed using STATA version 12 (STATA, College Station, Texas, USA). All applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during this research. Informed written consent was obtained from all participants, while the Ethical Committee of the Ahvaz Jundis-

hapur University of Medical Sciences approved this study.

## Results

Mean age of the participant was 60.6 (14.0) years. ESRD was documented in 15 out of 40 participants (38.5%). Baseline characteristics of participants have been presented in table 1. The median and IQR of serum and of salivary $\beta_2$M were 9.20 (0.44) and 6.23 (3.78), respectively. All patients were at taking either angiotensin converting enzyme inhibitor or angiotensin II receptor antagonist.

As shown in the table 2, none of the studied variables were associated with serum $\beta_2$M, while showing a p>0.1.

*Table 1: Baseline characteristics of participants*

| Variable | Median (IQR) |
|---|---|
| **Age (Y)** | 61.00 (13.50) |
| **Height (cm)** | 170.00 (4.00) |
| **Weight (kg)** | 80.00 (19.00) |
| **Body mass index ($kg.m^{-2}$)** | 27.07 (6.32) |
| **Blood urea nitrogen ($mg.dl^{-1}$)** | 48.00 (38.00) |
| **Serum creatinine ($mg.dl^{-1}$)** | 3.30 (5.55) |
| **Serum $\beta_2$M ($mg.dl^{-1}$)** | 9.20 (0.44) |
| **Salivary urea nitrogen ($mg.dl^{-1}$)** | 13.00 (43.00) |
| **Salivary creatinine ($mg.dl^{-1}$)** | 0.25 (0.40) |
| **Salivary $\beta_2$M ($mg.dl^{-1}$)** | 6.23 (3.78) |

*Table 2: Association of $\beta_2$M with different variables*

| Variable | Regression coefficient (β)[1] | SE | Z | P value | 95% CIs | |
|---|---|---|---|---|---|---|
| **Body mass index ($kg.m^{-2}$)** | 0.70 | 1.56 | 0.44 | 0.663 | -2.47 | 3.86 |
| **Serum urea nitrogen (mg/dl)** | 7.62 | 10.30 | 0.74 | 0.461 | -13.27 | 28.50 |
| **Serum creatinine (mg/dl)** | 1.45 | 1.09 | 1.34 | 0.194 | -0.75 | 3.66 |
| **Salivary urea nitrogen (mg/dl)** | 4.43 | 9.71 | 0.46 | 0.651 | -15.25 | 24.11 |
| **Salivary creatinine (mg/dl)** | 0.09 | 0.12 | 0.77 | 0.453 | -0.15 | 0.34 |
| **Salivary $\beta_2$M (mg/dl)** | 0.71 | 0.71 | 1.01 | 0.322 | -0.72 | 2.15 |

***Effect size denotes age-adjusted effect of a one-unit increase in $\beta_2$-Microglobulin ($\beta_2$M) on varying variables. It was obtained from age-adjusted linear regression models (coefficient of regression).***

The Spearman's ρ for correlation between serum and salivary $\beta_2$M was -0.017 (p=0.917), indicating lack of correlation. We also investigated the nonlinear association between serum and salivary $\beta_2$M and found no evidence of nonlinearity as is depicted in the figure 1.

Serum and salivary creatinine (Spearman's ρ=0.54; p<0.001) as well as serum and salivary urea nitrogen levels (Spearman's ρ=0.39; p=0.014)) were correlated.

Increasing concentrations of serum $\beta_2$M (RR=1.44, 95% CIs: 0.41-5.05; p=0.571) and salivary $\beta_2$M (RR=1.06, 95% CIs: 0.82-1.36; p=0.671) were associated with non-significant increased risk of ESRD. We also investigated the nonlinear association between serum and salivary $\beta_2$M. The incorporating natural cubic spline functions of the Salivary $\beta_2$ Macroglobulin (nonlinear linear term only (likelihood ratio test $\chi^2$=5.4; p=0.134). As shown in the table 3, it is evident that there are no significant improvements in the values of AIC.

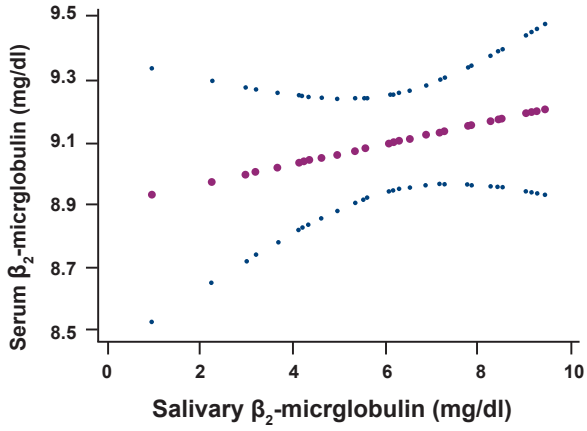


*Fig 1: The nonlinear association between serum and salivary $\beta_2$M.*

*Table 3: Nonlinear versus linear association between salivary $\beta_2$ macroglobulin and end stage renal disease*

| | | β coefficient | SE | Z | P value | 95% CIs | |
|---|---|---|---|---|---|---|---|
| **Nonlinear model** | | | | | | | |
| **Salivary $\beta_2$ macroglobulin** | Standardized function | 0.09 | 0.08 | 1.16 | 0.26 | -0.07 | 0.25 |
| | Orthogonalized basis 1 | 0.10 | 0.08 | 1.30 | 0.20 | -0.06 | 0.26 |
| | Orthogonalized basis 2 | -0.05 | 0.08 | -0.65 | 0.52 | -0.21 | 0.11 |
| | Orthogonalized basis 3 | 0.14 | 0.08 | 1.76 | 0.09 | -0.02 | 0.30 |
| | Age (Y) | 0.01 | 0.01 | 1.24 | 0.22 | 0.00 | 0.02 |
| | Intercept | 8.65 | 0.37 | 23.26 | 0.00 | 7.90 | 9.41 |
| **Akaike information criteria** | 61.19 | | | | | | |
| **Deviance** | 49.2 | | | | | | |
| **Linear model** | | | | | | | |
| **Salivary $\beta_2$ macroglobulin** | Linear function | 0.04 | 0.04 | 1.01 | 0.32 | -0.04 | 0.11 |
| | Age (Y) | 0.00 | 0.01 | 0.67 | 0.51 | -0.01 | 0.02 |
| | Intercept | 8.63 | 0.47 | 18.54 | 0.00 | 7.69 | 9.58 |
| **Akaike information criteria** | 60.77 | | | | | | |
| **Deviance** | 54.8 | | | | | | |
| **Nonlinear vs. linear likelihood ratio test χ2 (p value)** | 5.6 (0.134) | | | | | | |

## Discussion

Using a blood sample of adult diabetic men with CKD, we examined the concordance between serum and salivary $\beta_2$M and observed that salivary $\beta_2$M levels poorly agreed with serum $\beta_2$M levels, and thus may not be used as a surrogate for serum $\beta_2$M. We observed, however, a moderate correlation between serum and salivary levels of creatinine and urea.

Our finding of interest was that both salivary and serum levels of $\beta_2$M predicted the presence of ESRD, although the contributions failed to achieve statistical significance. The degree of accumulation of $\beta_2$M in patients undergoing hemodialysis have been previously observed to depend on the loss of renal excretory function (33). In patients with chronic renal failure, $\beta_2$M levels have been observed to parallel an increase in serum creatinine. A dramatic decrease in beta $\beta_2$M levels have been reported to be correlated with improvement in GFR (34).

Zhang et al. (35) have argued that for clinical applications, such as monitoring health status, disease onset and progression, and treatment outcome, there are following three necessary prerequisites:

1.A simple method for collecting biologic samples, ideally noninvasively.
2.Specific biomarkers associated with health or disease.
3.A technology platform to rapidly utilize the biomarkers.

$\beta_2$M has been demonstrated to be a major prognosticator of mortality in hemodialytic patients, independent of hemodialysis length, diabetes, malnutrition and chronic inflammation, suggesting the clinical importance of lowering and periodical monitoring of serum $\beta_2$M in these patients (36). However, venipuncture of the patients with CKD is an exhausting task to accomplish both for patients and health care providers. It is a highly desired skill in health care promotion and delivery to monitor health status, disease onset and progression, and treatment outcome through nonaggressive methods. Saliva is considered a complete medium to be explored for health and disease inspection (37). Saliva is appealing in that thereof taking samples does not require invasive procedure. It is commonly considered as the 'mirror of the body', and can be a perfect alternate method for clinical diagnostic (35). Utilizing easily accessible saliva for evaluating CKD may enable frontline care providers to become more involved and proactive in the management of CKD, facilitating a new way in order to focus on early detection and targeted interventions of vulnerable persons.

Achieving the goal of salivary diagnostics needs two prerequisites to be completed first:

1. Identification of specific biomarkers associated with a health or disease state.
2. The development of technologies that can discriminate between the biomarkers.

Recently, National Institute of Dental and Craniofacial Research has set a goal of using saliva as the diagnostic medium to evaluate the health or disease status of patients. This attempt could be looked upon as an ideal opportunity to optimize state-of-the-art saliva-based biosensors for salivary biomarkers that discriminate between diseases (37).

We observed a moderate correlation between serum and salivary levels of creatinine and urea. The concentration of salivary creatinine has been documented to be 10-15% of serum creatinine concentrations in healthy people. It has been, however, argued that this proportion may not hold among patients with renal disease. It has been shown that salivary creatinine estimations may be used to identify subjects with serum creatinine concentrations above 120 mmol.l-1 (38). Goll and Mookerje have pointed that "in hemodialysis patients, concentration of serum creatinine and uric acid is correlated with those in simultaneously drawn unstimulated whole saliva before and after dialysis" (39). The same findings have also been observed among patients with moderate renal failure not requiring the chronic hemodialysis. Use of whole saliva in this setting may preclude the iatrogenic component in anemia by cutting down the frequency of venipuncture; this could be of greater importance to young patients. On the other hand, it has been argued that salivary composition in patients with CKD varies by the stage of renal failure (40).

### *Limitations*

We did not use immunoassay for measurement

of $\beta_2$M. Furthermore, levels of serum $\beta_2$M did not vary among participants in the current study. We failed to demonstrate any association between increased levels of serum or salivary $\beta_2$M and ESRD. Wide confidence intervals indicated that our sample size probability did not have enough statistical power to capture the trivial associations observed.

## Conclusion

Using a blood sample of adult diabetic men with CKD, we examined the concordance between serum and salivary $\beta_2$M and observe that salivary $\beta_2$M levels poorly agreed with serum $\beta_2$M levels, and thus may not be used as a surrogate for serum $\beta_2$M in this highly selected subgroup of patients. Future prospective studies with larger sample size will be required to investigate whether $\beta_2$M can predict development of ESRD or not. We observed, however, a moderate correlation between serum and salivary levels of creatinine and urea. The clinical relevance of these findings remains to be illustrated.

## Acknowledgments

The authors express their gratitude to the participants for their time and patience. We are also grateful to the anonymous referees for their thoughtful and constructive comments. Special thanks to Molook Salemzadeh, Clinical Development Research Center of Golestan Hospital, for methodology and submission consultation, as well as Minoo Baratifar for data collection. The authors have no conflict of interest in this article.

## References

1. Hamer RA, El Nahas AM. The burden of chronic kidney disease. BMJ. 2006; 332(7541): 563-564.
2. 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(2): 137-147.
3. Sarnak MJ, Levey AS, Schoolwerth AC, Coresh J, Culleton B, Hamm LL, et al. Kidney disease as a risk factor for development of cardiovascular disease: a statement from the American Heart Association Councils on Kidney in Cardiovascular Disease, High Blood Pressure Research, Clinical Cardiology, and Epidemiology and Prevention. Circulation. 2003; 108(17): 2154-2169.
4. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care. 2004; 27(5): 1047-1053.
5. Weiner DE, Tighiouart H, Amin MG, Stark PC, MacLeod B, Griffith JL, et al. Chronic kidney disease as a risk factor for cardiovascular disease and all-cause mortality: a pooled analysis of community-based studies. J Am Soc Nephrol. 2004; 15(5): 1307-1315.
6. Shulman NB, Ford CE, Hall WD, Blaufox MD, Simon D, Langford HG, et al. Prognostic value of serum creatinine and effect of treatment of hypertension on renal function. Results from the hypertension detection and follow-up program. The Hypertension Detection and Follow-up Program Cooperative Group. Hypertension. 1989; 13 Suppl 5: I80-I93.
7. Culleton BF, Larson MG, Wilson PWF, Evans JC, Parfrey PS, Levy D. Cardiovascular disease and mortality in a community-based cohort with mild renal insufficiency. Kidney Int. 1999; 56(6): 2214-2219.
8. Shlipak MG, Simon JA, Grady D, Lin F, Wenger NK, Furberg CD. Renal insufficiency and cardiovascular events in postmenopausal women with coronary heart disease. J Am Coll Cardiol. 2001; 38(3): 705-711.
9. Sarnak MJ, Levey AS. Cardiovascular disease and chronic renal disease: a new paradigm. Am J Kidney Dis. 2000; 35(4 Suppl 1): S117-S131.
10. Parfrey PS, Foley RN. T The clinical epidemiology of cardiac disease in chronic renal failure. J Am Soc Nephrol. 1999; 10(7): 1606-1615.
11. Levey AS, Beto JA, Coronado BE, Eknoyan G, Foley RN, Kasiske BL, et al. Controlling the epidemic of cardiovascular disease in chronic renal disease: what do we know? What do we need to learn? Where do we go from here?. National Kidney Foundation Task Force on Cardiovascular Disease. Am J Kidney Dis. 1998; 32(5): 853-906.
12. McCullough PA. Why is chronic kidney disease the "spoiler" for cardiovascular outcomes?. J Am Coll Cardiol. 2003; 41(5): 725-728.
13. Schwalbe S, Holzhauer M, Schaeffer J, Galanski M, Koch KM, Floege J. Beta 2-microglobulin associated amyloidosis: a vanishing complication of long-term hemodialysis?. Kidney Int. 1997; 52 (4): 1077-1083.
14. Vincent C, Chanard J, Caudwell V, Lavaud S, Wong T, Revillard JP. Kinetics of 125I-beta 2-microglobulin turnover in dialyzed patients. Kidney Int. 1992; 42 (6): 1434-1443.
15. Astor BC, Shafi T, Hoogeveen RC, Matsushita K, Ballantyne CM, Inker LA, et al. Novel markers of kidney function as predictors of ESRD, cardiovascular disease, and mortality in the general population. Am J Kidney Dis. 2012; 59(5): 653-662.
16. Saijo Y, Utsugi M, Yoshioka E, Horikawa N, Sato T, Gong Y, et al. Relationship of beta2-microglobulin to arterial stiffness in Japanese subjects. Hypertens Res. 2005; 28(6): 505-511.
17. Zumrutdal A, Sezer S, Demircan S, Seydaoglu G, Ozdemir FN, Haberal M. Cardiac troponin I and beta 2 microglobulin as risk factors for early-onset atherosclerosis in patients on haemodialysis. Nephrology (Carlton). 2005; 10(5): 453-458.
18. Wilson AM, Kimura E, Harada RK, Nair N, Narasimhan B, Meng XY, et al. Beta2-microglobulin as a biomarker in peripheral arterial disease: proteomic profiling and clinical studies. Circulation. 2007; 116(12): 1396-1403.
19. Amighi J, Hoke M, Mlekusch W, Schlager O, Exner M, Haumer M, et al. Beta 2 microglobulin and the risk for cardiovascular events in patients with asymptomatic carotid atherosclerosis. Stroke. 2011; 42(7): 1826-1833.
20. Liabeuf S, Lenglet A, Desjardins L, Neirynck N, Glorieux G, Lemke HD, et al. Plasma beta-2 microglobulin is associated with cardiovascular disease in uremic patients. Kidney Int. 2012; 82(12): 1297-1303.
21. Okuno S, Ishimura E, Kohno K, Fujino-Katoh Y, Maeno Y, Yamakawa T, et al. Serum beta2-microglobulin level is a significant predictor of mortality in maintenance haemodialysis patients. Nephrol Dial Transplant. 2009; 24(2):

571-577.
22. Slavkin HC. Toward molecularly based diagnostics for the oral cavity. J Am Dent Assoc. 1998; 129(8): 1138-1143.
23. Streckfus CF, Bigler LR. Saliva as a diagnostic fluid. Oral Dis. 2002; 8(2): 69-76.
24. Arici M. 'Ideal criteria' for starting chronic hemodialysis: numbers, symptoms or an alerting 'traffic light' system? Nephron Clin Pract. 2012; 120(1): c17-24.
25. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron. 1976; 16(1): 31-41.
26. Levey AS, Eckardt KU, Tsukamoto Y, Levin A, Coresh J, Rossert J, et al. Definition and classification of chronic kidney disease: a position statement from Kidney Disease: Improving Global Outcomes (KDIGO). Kidney Int. 2005; 67(6): 2089-2100.
27. Hsu CY, Chertow GM. Chronic renal confusion: insufficiency, failure, dysfunction, or disease. Am J Kidney Dis. 2000; 36(2): 415-418.
28. Berti-Couto Sde A, Couto-Souza PH, Jacobs R, Nackaerts O, Rubira-Bullen IR, Westphalen FH, et al. Clinical diagnosis of hyposalivation in hospitalized patients. J Appl Oral Sci. 2012; 20(2): 157-161.
29. Harrell FE. Spingers Series in Statistics: Regression modeling strategies. New York: Springer; 2001; 19-20.
30. Akaike H. A new look at the statistical model identification. IEEE Trans Automat Contr. 1974; 19(6): 716-723.
31. Cummings P. The relative merits of risk ratios and odds ratios. Arch Pediatr Adolesc Med. 2009; 163(5): 438-445.
32. Zhang J, Yu KF. What's the relative risk?. A method of correcting the odds ratio in cohort studies of common outcomes. JAMA. 1998; 280(19): 1690-1691.
33. Floege J, Bartsch A, Schulze M, Shaldon S, Koch KM, Smeby LC. Clearance and synthesis rates of beta 2-microglobulin in patients undergoing hemodialysis and in normal subjects. J Lab Clin Med. 1991; 118(2): 153-165.
34. Acchiardo S, Kraus AP Jr, Jennings BR. Beta 2-microglobulin levels in patients with renal insufficiency. Am J Kidney Dis. 1989; 13(1): 70-74.
35. Zhang L, Xiao H, Wong DT. Salivary biomarkers for clinical applications. Mol Diagn Ther. 2009; 13(4): 245-259.
36. Okuno S, Ishimura E, Kohno K, Fujino-Katoh Y, Maeno Y, Yamakawa T, et al. Serum β2-microglobulin level is a significant predictor of mortality in maintenance haemodialysis patients. N Nephrol Dial Transplant. 2009; 24(2): 571-577.
37. Wong DT. Salivary diagnostics powered by nanotechnologies, proteomics and genomics. J Am Dent Assoc. 2006; 137(3): 313-321.
38. Lloyd JE, Broughton A, Selby C. Salivary creatinine assays as a potential screen for renal disease. Ann Clin Biochem. 1996; 33 ( Pt 5): 428-431.
39. Goll RD, Mookerjee BK. Correlation of biochemical parameters in serum and saliva in chronic azotemic patients and patients on chronic hemodialysis. J Dial. 1978; 2(4): 399-344.
40. Tomás I, Marinho JS, Limeres J, Santos MJ, Araújo L, Diz P. Changes in salivary composition in patients with renal failure. Arch Oral Biol. 2008; 53(6): 528-532.