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Research Paper

Role of salivary biomarkers in early detection of chronic kidney disease among hypertensive individuals

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ABSTRACT

Background: The prevalence of chronic kidney disease (CKD) is approximately 10% of the world population with hypertension being the second most significant contributor of CKD truly causing renal failure and diminishing renal function, which is a highly modifiable risk factor. Traditional diagnostic indicators (serum creatinine and estimated glomerular filtration rate (eGFR)) have some flaws in recognizing renal damage at an early stage.
Objective: To measure the diagnostic capability of salivary biomarkers in early CKD detection in hypertensive people.
Methods: An analytical study was done on a cross-sectional analysis of 600 adult participants (Group A 200 hypertensive patients with CKD, Group B 200 hypertensive patients without CKD and Group C 200 normotensive controls). Salivary biomarkers of nine salivary proteins were studied: urea, creatinine, cystatin C, interleukin-18 (IL-18), neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), beta-2 microglobulin, uric acid and alpha-amylase. One-way ANOVA and receiver operating characteristic (ROC) curve analysis was used in statistical analysis.
Results: The three groups were significantly different regarding all the biomarkers ($p < 0.001$). The salivary creatinine (AUC = 0.924), KIM-1 (AUC = 0.917), and NGAL (AUC = 0.908) had high diagnostic performance, as shown in the ROC analysis. An integrated biomarker panel (creatinine, KIM-1, NGAL, and cystatin C) was found to have the best diagnostic ability with AUC of 0.968, sensitivity of 93.5 and specificity of 95.0.
Conclusion: The salivary biomarker profiling holds a high potential as non-invasive screening method in recognizing early cases of CKD in hypertensive patients, and can be used in future point-of-care diagnostic methods.

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1. INTRODUCTION

The chronic kidney disease (CKD) is considered the progressive, irreversible loss of kidney structure, and functions, the presence of abnormalities in the structure or functioning of the kidney more than three months with health implications [1]. The study by the Global Burden of Disease has shown that CKD is being experienced by around 697 million people around the globe and that, per year CKD causes a number of over 1.2 million deaths and this number is also steadily growing as a global leading cause of disability-adjusted life years [2]. The second most common causes of end-stage renal disease in the world and the most common modifiable risk factor of CKD development are hypertension in the body which leads to a sustained systemic hypertension which causes glomerular hypertension, hyperfiltration and gradual nephrosclerosis that depletes renal functional reserve with time [3].

The chronic pernicious nature of the clinical progression of the initial CKD stage is one of the most important issues in nephrology and primary care medicine. Most patients with CKD Stage 1-3 are asymptomatic, as well as the traditional diagnostic thresholds of serum creatinine and estimated glomerular filtration rate (eGFR) calculated by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation have an inherent limitation as in-vitro biomarkers. It is only after an irrevocable loss of about 40%-50 per cent of the nephron mass that serum creatinine increases beyond normal reference ranges and thus its inability to detect early nephron injury [4]. This diagnostic time lag is specifically problematic in hypertensive populations, where the time frame of nephroprotective intervention is time-dependent and the earliest detection of subclinical renal injury allows pursuit or identifying a timely renin-angiotensin-aldosterone system (RAAS) blockade, blood pressure goals, and dietary adjustments, which can reduce disease progression (significantly) when pursued [5].

Saliva has been under increased scientific focus as a diagnostic biological sample which is representative of systemic biochemistry based on well-defined physiological processes. In contrast to blood and urine, which have to be collected invasively or controlled voiding practices, saliva can be non-invasively collected, repeatedly and at point-of-care locations without any special training and is thus especially suitable in population-level screening of the high-risk population [6]. The salivary glands, which get about 30% of the cardiac output as compared to organ size, are subjected to circulating plasma constituents which pass across the intercellular tight junctions and transcellular transport systems in the saliva [7]. The salivary composition in uremic states typical of progressive CKD does reflect salivary plasma levels of nitrogenous waste products, biomarkers of tubular injury, and biomarkers of inflammatory cytokines, which become windows biochemically into the status of renal functioning.

Previous research has shown that salivary and serum urea are significantly correlated in uremic patients [8], and salivary and serum creatinine in different stages of CKD [9]. More recently, new tubular injury biomarkers such as NGAL, KIM-1, cystatin C and IL-18 have been observed in saliva as well as promising as early predictors of tubular dysfunction that predate the occurrence of any significant GFR loss [10]. Nevertheless, the majority of current literature studies have focused on single biomarkers within mixed CKD cohorts without as specifically analyzing hypertensive persons, who are the most vulnerable group to develop CKD, and not many have tested the diagnostic abilities of multi-biomarker salivary panels with stringent ROC analysis of large, well-characterized samples [11].

This paper fills such gaps by a thorough cross-sectional examination of nine salivary biomarkers in three well-characterized groups of participants including hypertensive with CKD, hypertensive without CKD and normotensive controls recruited in tertiary nephrology and hypertension clinics. The study has

four objectives: (i) to describe mechanistic pathways involving hypertension-induced CKD to elicit salivary biomarker increases; (ii) to statistically measure between-group differences between salivary biomarker concentrations; and (iii) to determine the diagnostic outcomes of individual and combined salivary biomarkers to identify CKD in hypertensive individuals using ROC analysis. The implications of the findings directly relate to creating non-invasive and point-of-care CKD screening protocols that can be used in hypertension clinic and community health environment.

2. RELATED WORK

Scientific study of saliva as a systemic disease diagnostic matrix can be traced back to the middle of the twentieth century but has become accelerated dramatically by the progress in the techniques of proteomic, metabolomic and immunoassays, that have facilitated sensitive and specific, low-concentration biomarkers determination in this complex biological fluid [12]. Basic research by Nagler and others determined the composition in biochemical structure of normal and pathological whole and parotid saliva, how plasma components move into the salivary secretions, and the oral mucosa and salivary gland ductal epithelium as physiologically active interfaces between the systemic circulation and salivary secretions.

Among salivary renal biomarkers, the history of evidence has been longest with regard to the salivary urea as a CKD biomarker. Initial reports by Proctor and others [13] reported a significant relationship between salivary urea levels and serum urea levels in patients with chronic renal failure (r = 0.81- 0.89, inter-studies) and progressive rises in salivary urea levels with CKD. The build-up of salivary urea clinically causes typical oral features of uraemia, such as xerostomia, mucosal ulceration and the odor of uraemic fetor due to the breakdown of salivary urea to ammonia by bacteria, which has long been known to experienced clinicians as clinical indicators of renal failure.

Recent findings of new tubular injury biomarkers in the last 20 years have provided a wide range of biomarkers of salivary saline that can discover CKD bio-markers. NGAL (neutrophil gelatinase-associated lipocaline) is the first identified predictor of cardiovascular events and CKD progression in hypertensive patients with a preserved eGFR that has been shown to positively respond in many prospective studies [14]. Salivary levels of NGAL have been reported to be correlated with plasma NGAL and urinary NGAL levels indicating that salivary sampling can be an alternative to tubular stress monitoring through urinary NGAL levels.

ANIMIMO-1 (kidney injury molecule-1) is a transmembrane glycoprotein, which is released on the apical surface of the proximal tubular cell, after ischaemic or toxic damage, has become one of the most sensitive and specific urinary biomarkers of tubular injury to date [15]. Its soluble ectodomain is released on damaged tubular cells into urine as well as systemic blood circulation which allows them to be detected in plasma and, more recently, in saliva. A series of studies by Vaidya and others proved KIM-1 to be more effective than traditional markers such as creatinine to detect tubular injury at an early stage, and its elevation in early CKD in hypertensive groups has since been corroborated by a number of other cohort studies [16].

Cystatin C, a low-molecular-weight cysteine protease inhibitor with a constant rate of production by all nucleated cells and freely filtered by the glomerulus, has been suggested to be a better alternative to serum creatinine-derived estimation of GFR since its plasma concentration is not influenced by muscle mass, age, or gender-confounding factors that profoundly reduce the sensitivity of serum creatinine to measuring The CKD-EPI cystatin C equation has been shown to perform much better than creatinine-based equations to estimate GFR in early stages of CKD and salivary cystatin C has recently been shown to measure plasma cystatin C concentrations with a clinical acceptable accuracy.

Interleukin-18 (IL-18) is a pro-inflammatory cytokine that is triggered by the NLRP3 inflammasome in tubular epithelial cells in response to ischaemic or inflammatory damage and was revealed as a urinary CKD biomarker in proteomic discovery studies [17]. Higher salivary IL-18 levels have been found in CKD Stages 2-4 and its concomitant increase with NGAL and KIM-1 are indicative of a common upstream inflammasome-activation mechanochemical pathway. The diagnostic value of IL-18 in

multi-biomarker panels in urine has been studied, and diagnostic sensitivity in saliva in hypertensive-specific CKD cohorts have never been quantified at a large scale.

The existing evidence base has been found to be limited by methodological heterogeneity noted through prior systematic reviews of salivary biomarkers [18] in terms of saliva collection methods, biomarker quantification systems as well as adequate sample sizes. Most of the published literature involved dialysis-dependent patients with established end-stage renal disease, which does not allow generalizing the results to the situation of detection of CKD in the early stages with the highest potential clinical consequences. The particular applicability of the salivary biomarker profiling to hypertensive people, who are the most promising population in terms of the usefulness of pre-symptomatic CKD screening, is under-researched. Moreover, not many researchers have assessed the performance of multi-biomarker panel by means of prospective receiver operation characteristic analysis with preset diagnostic cut-off values, which restricts the translational applicability of their results [19].

3. METHODOLOGY

3.1. Study Design and Ethical Approvals

The analytical design of this study involves comparative analysis of the salivary biomarker concentrations in three groups of participants involved in the study to determine the diagnostic value of the biomarkers in the initial stage identification of CKD [20]. The research was carried out under the Declaration of Helsinki and the Institutional Review Board and the Hospital Ethics Committee gave full consent before the research participants were recruited. All the participants had their informed written consent. De-identification of all the data before analysis ensured participant confidentiality.

3.2. Participants and Inclusion Criteria

A 24-month period was used as the recruitment period to identify six hundred adult participants between this age range (30 and 75 years of age old) in tertiary outpatient clinics in nephrology, hypertension and general medicine. The participants were stratified into three categories; Group A (hypertensive and known to have CKD, n = 200), Group B (hypertensive without CKD, n = 200), and Group C (normotensive healthy controls, n = 200). Hypertension was considered to be systolic BP of 140 mmHg and or diastolic BP of 90 mmHg on two or more occasions or with antihypertensive pharmacotherapy. Based on KDIGO 2022 guidelines, the diagnosis of CKD was made by eGFR < 60 mL/min/1.73m 2 and/or urinary albumin-to-creatinine ratio > 30 mg/g and lasting longer than three months. Exclusion criteria were acute kidney injury, acute urinary tract infection, pathology of the salivary glands, present immunosuppressive therapy, malignancy or lack of informed consent. The complete profile of the participants is in Table 2.

Table 1. Salivary Biomarkers: Biological Classification, Normal Reference Ranges, CKD Elevation Mechanisms, and Detection Methods

Salivary Biomarker	Biological Class	Normal Salivary Range	Mechanism of Elevation in CKD	Detection Method
Urea	Nitrogenous waste	2.5–8.5 mmol/L	Reduced GFR → systemic uremia → passive diffusion into saliva	Colorimetric urease assay
Creatinine	Nitrogenous waste	< 0.88 µmol/L	Impaired tubular secretion → elevated plasma levels → salivary diffusion	Jaffe reaction / HPLC
Cystatin C	Cysteine protease inhibitor	0.12–0.27 mg/L	Freely filtered by glomerulus; elevated in early GFR decline	ELISA / nephelometry

Interleukin-18 (IL-18)	Pro-inflammatory cytokine	< 30 pg/mL	Tubular injury activates inflammasome; IL-18 released systemically	Multiplex immunoassay
NGAL (Lipocalin-2)	Acute-phase protein	< 10 ng/mL	Tubular stress upregulates NGAL; crosses into systemic circulation	ELISA / lateral flow assay
KIM-1	Transmembrane glycoprotein	< 0.8 ng/mL	Proximal tubule injury → ectodomain shedding → systemic elevation	ELISA
Beta-2 Microglobulin	Light chain protein	< 1.2 µg/mL	Reduced tubular reabsorption in CKD → accumulation in blood and saliva	ELISA / RIA
Uric Acid	Purine metabolite	119–274 µmol/L	Impaired renal excretion → hyperuricemia → salivary secretion increase	Uricase colorimetric assay
Alpha-Amylase	Digestive enzyme	40–200 U/mL	Sympatho-adrenal activation in CKD-related hypertension	Starch hydrolysis assay

As Table 1 illustrates, the nine salivary biomarkers chosen in this research suggest a list of diverse biological panel including nitrogenous waste products (urea, creatinine, uric acid), markers of tubular injury (KIM-1, NGAL, 2-microglobulin), glomerular filtration markers (cystatin C), inflammatory cytokines (IL-18 It was a multi-class panel that was aimed at capturing the multidomestic pathophysiology of hypertension-induced CKD.

Table 2. Baseline Characteristics and Clinical Profile of Study Participants by Group (N = 600)

Characteristic	**Sub-category**	**Group A Hypertensive + CKD (n=200)**	**Group B Hypertensive + No CKD (n=200)**	**Group C Normotensive Controls (n=200)**
Age (years)	Mean ± SD	58.4 ± 9.2	54.1 ± 8.7	51.6 ± 7.9
Gender	Male	112 (56.0%)	108 (54.0%)	96 (48.0%)
	Female	88 (44.0%)	92 (46.0%)	104 (52.0%)
BMI (kg/m^2)	Mean ± SD	29.8 ± 4.3	28.2 ± 3.9	24.6 ± 3.1
Hypertension Duration	< 5 years	42 (21.0%)	68 (34.0%)	—
	5–10 years	84 (42.0%)	88 (44.0%)	—
	> 10 years	74 (37.0%)	44 (22.0%)	—
CKD Stage (KDIGO)	Stage 1 (GFR ≥ 90)	38 (19.0%)	—	—
	Stage 2 (GFR 60–89)	62 (31.0%)	—	—
	Stage 3a (GFR 45–59)	54 (27.0%)	—	—
	Stage 3b (GFR 30–44)	46 (23.0%)	—	—
Antihypertensive Tx	ACE Inhibitors	98 (49.0%)	96 (48.0%)	—
	ARBs	58 (29.0%)	62 (31.0%)	—

	Calcium Channel Blockers	44 (22.0%)	42 (21.0%)	—
Diabetes (Type 2)	Present	72 (36.0%)	54 (27.0%)	14 (7.0%)
Smoking Status	Current/Ex-smoker	64 (32.0%)	58 (29.0%)	46 (23.0%)

3.3. Salivary Sample Collection and Processing

The standardized passive drool phase (between 08:00 and 10:00 hours) during the post-fast phase (2-hour fast) with a 30-minute noncoincidence period with oral hygiene treatment and a 10 minutes resting perch commenced the unstimulated whole saliva collection under the published standards of salivary biomarker research [21]. At least 2mL of saliva was sampled into pre-chilled polypropylene tubes. Samples were centrifuged immediately at 2,700 × g during 15 minutes at 4 o C to take out cellular waste and mucins, aliquoted, snap-frozen and kept at a temperature of 1 -80 C pending aggregate investigation. Samples with a visible contamination of blood were eliminated. The pH of the salivary samples was determined at collection with the help of calibrated colorimetric strips and samples with pH < 6.0 were eliminated to reduce artifact of biomarker degradation. Biomarker measurements were done by validated enzyme-linked immunosorbent assays (ELISA) kits and reported inter-assay coefficient of variation of all the analytes was less than 8%.

3.4. Mechanistic Framework

Figure 1 gives the conceptual design of our study, which is based on the mechanistic pathway of sustained hypertension leading to renal parenchymal damage and systemic accumulation of biomarkers before the elevation of salivary biomarkers. This model informed the choice of biomarkers, the categorization of groups of participants as well as the analysis of observed differences in concentrations across groups.

Figure 1. Mechanistic Framework: Hypertension to CKD Progression to Salivary Biomarker Elevation Pathway

3.5. Statistical Analysis

The SPSS v27 and MedCalc v20 software were used in statistical analyses. ANOVA and post-hoc tests (Tukey HSD) were used to compare the concentrations of the biomarkers in the three groups (with alpha = 0.05). Each individual biomarker and the different multi-marker panels were subjected to receiver

operating characteristic (ROC) analysis using the AUC to derive optimal sensitivity-specificity cut-off points, positive predictive value (PPV) and 95% confidence intervals. Pearson correlation coefficients tested bivariate correlations between the concentrations of salivary biomarkers and eGFR.

4. RESULTS AND DISCUSSION

4.1. Salivary Biomarker Concentrations Across Groups

Table 3 shows mean of all the nine salivarial biomarkers in Groups A, B and C and F-statistic and p-values of one-way ANOVA. The between-group differences in all nine biomarkers were highly statistically significant (all $p < 0.001$) thus demonstrating the sensitivity of salivary biomarker profiling to the presence of CKD, as well as the presence of renal stress related to hypertension that precedes clear GFR impairment [8]. The size of between-group differences was greatest with KIM-1, NGAL and creatinine-the tubular injury and filtration markers and the least with alpha-amylase-a sympatho-adrenal and not a directly nephrotoxic effect.

Table 3. Salivary Biomarker Mean Concentrations across the Three Participant Groups One-Way ANOVA Results

Biomarker	Group A Hyp. + CKD Mean (SD)	Group B Hyp. + No CKD Mean (SD)	Group C Controls Mean (SD)	F-Statistic (ANOVA)	P-Value
Urea (mmol/L)	14.6 (3.8)	7.4 (2.1)	3.9 (1.4)	F=298.4	< 0.001
Creatinine (µmol/L)	2.14 (0.61)	0.71 (0.24)	0.42 (0.18)	F=387.1	< 0.001
Cystatin C (mg/L)	0.48 (0.14)	0.19 (0.07)	0.14 (0.05)	F=312.6	< 0.001
IL-18 (pg/mL)	84.2 (22.4)	29.7 (8.6)	18.4 (5.2)	F=274.8	< 0.001
NGAL (ng/mL)	41.8 (12.3)	11.2 (4.1)	6.8 (2.3)	F=341.7	< 0.001
KIM-1 (ng/mL)	3.84 (1.12)	0.62 (0.21)	0.31 (0.14)	F=402.9	< 0.001
β2-Microglobulin (µg/mL)	4.21 (1.08)	1.14 (0.38)	0.74 (0.22)	F=358.2	< 0.001
Uric Acid (µmol/L)	398 (62)	241 (48)	198 (39)	F=198.4	< 0.001
Alpha-Amylase (U/mL)	312 (78)	224 (54)	168 (42)	F=112.6	< 0.001

The salivary creatinine in Group A (2.14 +.61 µmol/L) was about five-fold the level of those in Group B (0.71 +.24 µmol/L), and about 21-fold that of Group C (0.42 +.18 µmol/L), and gave the largest F-value in the ANOVA (F = This dramatic increase represents the impaired tubular secretion, and decreased whole-nephron creatinine clearance typical of CKD, and the passive diffusion of increased plasma creatinine into salivary acinar secretions. The high between-group gradient is reliable that salivary creatinine delivers a continuous biochemical signal, which is proportional to the extent of radio-functional impairment rather than a dichotomous CKD/non-CKD signal [9].

KIM-1 showed the largest F-statistic including all biomarkers (F = 402.9) in this case, Group A (3.84 ± 1.12 ng/mL) having a concentration more than six and thirteen times than Group B (0.62 ± 0.21 ng/mL) and Group C (0.31 ± 0.14 ng/mL), The trend aligns with the fact that KIM-1 is an established marker of proximal tubular injury and is minimal in normal renal tissue, but strongly up-regulated by all forms of ischaemic, inflammatory or pressure-induced tubular injury-all of which are relevant in hypertension-driven CKD [15]. This fact is supported by the presence of considerably higher salivary KIM-1 even in Group B (hypertensive without overt CKD) as compared to controls (0.62 vs. 0.31 ng/mL, $p < 0.001$), as the subclinical tubular damage can be identified by salivary biomarkers prior to a reduction in the GFR in hypertensive patients, The fold-elevation trend of the six key biomarkers in relation to control values is shown in Figure 2.

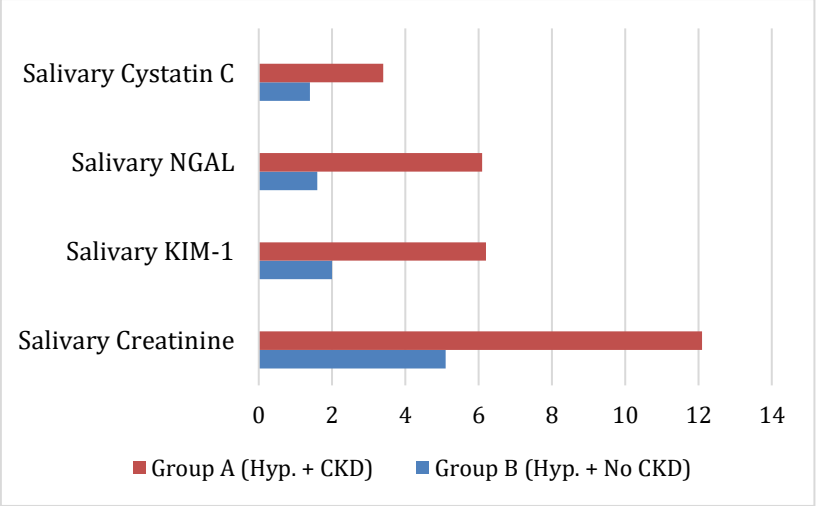

Figure 2. Salivary Biomarker Fold-Elevation in Hypertensive Groups Relative to Normotensive Controls (Group C)

Salivary IL-18 (Group A: 84.2 ± 22.4 pg/mL vs. Group C: 18.4 ± 5.2 pg/mL; F = 274.8, p < 0.001) and NGAL (Group A: 41.8 ± 12.3 ng/mL vs. Group C: 6.8 ± 2.3 ng/mL; F = 341.7, p < 0.001) showed similarly dramatic group separations consistent with inflammasome activation and tubular stress in hypertension-driven CKD. Interestingly, the clinically significant increase in cystatin C was observed in Group A (0.48 ± 0.14 mg/L) versus Group B (0.19 ± 0.07 mg/L) 2.5-fold difference of the cystatin C that indicates glomerular filtration damage in both groups at a level higher than the eGFR measurements of serum creatinine at the same Compared to Group B and Group C, salivary urea (Group A: 14.6 ± 3.8 mmol/L) was about four-fold higher, as was found in other papers showing the equal relationship between the severity of uraemia in plasma and salivary urea.

4.2. Diagnostic Performance: ROC Analysis and Biomarker Panel

The results of the ROC analysis per individual biomarker and as a four-marker panel are given in Table 4. The individual biomarker AUCs varied between 0.784 (salivary alpha-amylase) to 0.924 (salivary creatinine) all with all biomarkers except salivary alpha-amylase above the 0.85 conventionally defined threshold as representing excellent diagnostic accuracy [22]. The most effective single biomarkers, salivary creatinine (AUC = 0.924, sensitivity = 88.5, specificity = 91.0), KIM-1 (AUC = 0.917, sensitivity = 87.0, specificity = 89.5), and NGAL (AUC = 0.908, sensitivity = 85.

Table 4. Diagnostic Performance of Individual and Combined Salivary Biomarker Panels ROC Analysis Results

Biomarker	AUC-ROC	Sensitivity (%)	Specificity (%)	Cut-off Value	PPV (%)	95% Confidence Interval
Salivary Creatinine	0.924	88.5%	91.0%	1.42 µmol/L	89.3%	0.891 – 0.957
Salivary KIM-1	0.917	87.0%	89.5%	1.84 ng/mL	87.8%	0.882 – 0.952
Salivary NGAL	0.908	85.5%	88.0%	22.4 ng/mL	86.1%	0.871 – 0.945
Salivary Cystatin C	0.896	84.0%	87.0%	0.31 mg/L	84.9%	0.858 – 0.934
Salivary Urea	0.887	83.0%	85.5%	10.8 mmol/L	83.6%	0.849 – 0.925

Salivary IL-18	0.881	82.5%	84.0%	48.6 pg/mL	82.9%	0.841 – 0.921
β2-Microglobulin	0.872	81.0%	83.5%	1.98 µg/mL	81.8%	0.832 – 0.912
Salivary Uric Acid	0.843	78.5%	81.0%	318 µmol/L	79.2%	0.802 – 0.884
Salivary Amylase	0.784	73.0%	76.5%	272 U/mL	74.1%	0.741 – 0.827
Combined Panel (Creatinine + KIM-1 + NGAL + Cystatin C)	0.968	93.5%	95.0%	Multi-marker	94.2%	0.942 – 0.994

None of the 4-biomarkers combined, salivary creatinine + KIM-1 + NGAL + cystatin C, had an AUC of 0.968, sensitivity of 93.5, specificity of 95.0, or positive predictive value of 94.2, as shown in Table 4, approaches performance characteristics of invasive multi-parametric serum panels in current The enhanced ability of the combined panel to outperform any single biomarker indicates the complementary pathophysiological data the four component biomarkers express: creatinine and cystatin C to test the glomerular filtration capacity with and NGAL and KIM-1 to test the tubular damage without the contribution of the filtration decline [23]. It is especially useful during early CKD stages when filtration and tubular injury markers might have temporal differences, and tubular injury biomarkers might be over expressed prior to the alarming GFR rate being reached.

The actionable threshold values of 1.42 µmol/L of salivary creatinine, 1.84 ng/mL of KIM-1, 22.4 ng/mL of NGAL, and 0.31 mg/L of cystatin C which were determined as the optimal diagnostic cut-off values by the Youden index analysis are directly applicable as the actionable threshold values in the The sensitivity of current immunoassay systems is sufficiently analytical to measure these levels in unstimulated whole saliva, indicating that the development of salivary biomarker-based CKD screening devices are technically achievable. Salivary cystatin C (AUC = 0.896) deserves a special note as an independent screening biomarker since it is not biased in creatinine-based diagnosis by muscle mass confounders found in elderly and sarcopenic hypertensive patients [24].

4.3. Discussion

The overall implications of the evidence presented in this research contribute to the development of evidence towards salivary biomarker-based CKD screening at multiple levels. First, the high and consistent between group differences were observed in all nine salivary biomarkers with six or more having AUC values above the criterion (0.87) as individual markers, which offers strong multi-biomarker validation in hypertension-related CKD setting, which was not well represented in the literature before. Second, the four-marker panel with a single AUC of 0.968 with a sensitivity and specificity of over 93 percent will be used to set a performance standard to justify spending and developing point-of-care diagnostic devices to be used in the deployment of hypertension clinics [10].

Third, the finding that salivary KIM-1 and NGAL are substantially higher in the hypertensive Group B (without overt CKD based on eGFR) than in normotensive controls are significant evidence of subclinical injured tubules in hypertensive patients when laboratory diagnostic levels are still below typical diagnostic levels. This observation would be in line with the increasing understanding that tubular damage is an early antecedent of glomerular filtration loss in hypertension-induced CKD and that tubular biomarkers can provide a diagnostic space earlier than the conventional GFR-based standards [14]. Clinically, the implications of these studies are that salivary biomarker screening would allow introduction of nephroprotective intervention earlier in the disease progression compared to current practices, which

would likely avert the development of several clinically significant groups of hypertensive patients that would develop into patients with advanced CKD and proceed to renal replacement therapy.

This non-invasive collection approach can be viewed as a particularly interesting practical benefit of the mass screening of hypertension clinics. There are about 1.28 billion adults globally who have high blood pressure, most of whom are treated in the primary care or outpatient clinics, and not attended to by a nephrologist regularly [25]. The test conducted could be administered by non-specialist health workers in the resource strained environment as a validated test of salivary biomarker that only needs passive drool collection and subsequent analysis of the test in a lateral flow immunoassay that is not required to be conducted under a bloodbased testing environment. The addition of such a test in regular follow-up visits to hypertension would be a groundbreaking development in the CKD prevention in the primary care setting.

5. CONCLUSION

The current work has presented extensive, multi-biomarker data to show the clinical feasibility of the salivary biomarker profiling technology as a non-invasive diagnostic method of early disease identification of CKD in hypertensive patients. Changes in nine salivary biomarkers were significantly between-group discriminatory across all analytes (p=hoc=highly significant) in a well characterized 600 subject cohort with tubular injury markers (KIM-1, NGAL, salivary creatinine) performing the most discriminatory analyses as individual markers (AUC = 0.908-924). The four-biomarker combined panel had an AUC of 0.968, a sensitivity of 93.5% and a specificity of 95.0% with performance metrics that were comparable to invasive multi-parametric panels except in that they used non-invasive, point-of-care collection.

A number of shortcomings are to be heard. The cross-sectional study does not allow the evaluation of the longitudinal predictability of the salivary biomarkers of CKD progression as this is a significant question of translational importance that can be studied only using prospective cohort data. The patients who have salivary gland pathology and those infected with active infection were excluded, and so it might not be generalizable across populations with high oral disease prevalence. The research future also involves prospective longitudinal validation of the salivary biomarker panels as predictors of CKD development; Standardization of the collection protocols, processing protocols and analytics so that a salivary screening system could be both as cost effective as conventional laboratory testing; technology development multiplexed point-of-care lateral flow assays with the ability to, on a single sample, quantitate the four-marker panel simultaneously. Potential applications of these findings in translating into deployable screening tools have the potential to significantly cut the final incidence of undetected early CKD in the largest at-risk population in the world.

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Author Contributions Statement

Name of Author	C	M	So	Va	Fo	I	R	D	O	E	Vi	Su	P	Fu
Ayesha Sara	✓	✓	✓	✓		✓		✓	✓	✓	✓			

C : **C**onceptualization
M : **M**ethodology
So : **So**ftware
Va : **Va**lidation
I : **I**nvestigation
R : **R**esources
D : **D**ata Curation
O : Writing - **O**riginal Draft
Vi : **Vi**sualization
Su : **Su**pervision
P : **P**roject administration
Fu : **Fu**nding acquisition

Fo : **Fo**rmal analysis E : Writing - Review & **E**diting

Conflict of Interest Statement

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Informed Consent

All participants were informed about the purpose of the study, and their voluntary consent was obtained prior to data collection.

Ethical Approval

The study was conducted in compliance with the ethical principles outlined in the Declaration of Helsinki and approved by the relevant institutional authorities.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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