Original Article

Renal Resistive Index and activity of Renin-angiotensin-aldosterone System Components in Persian Cats With Polycystic Kidney Disease

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ABSTRACT

Background: Polycystic kidney disease (PKD) in Persian cats is a common genetic disorder that accounts for 10% of chronic renal failures.

Objectives: This study aimed to assess the effect of PKD progression on renal resistive index (RI), plasma renin activity (PRA), angiotensin II (ANG II), aldosterone levels, and systolic blood pressure in Persian cats.

Methods: Fifty Persian cats (25 with PKD and 25 healthy) were included in the present study. First, the blood pressure of each cat was measured, and then their PRA, ANG II, and aldosterone enzymes were evaluated using an ELISA test. Additionally, B-Mode ultrasonography was performed in the PKD group to evaluate and calculate cysts' diameter and overall volume (OVC). Furthermore, the RI was computed by pulsed-wave Doppler in all cats.

Results: There were no significant differences in the systolic blood pressure between healthy and PKD cats (138.84±2.89 vs 140.92±2.35 mm Hg). PRA, aldosterone, and ANG II were significantly higher in the PKD group compared to the healthy group (3.64±0.36 vs 2.26±0.029 ng/mL, P<0.01; 80.45±2.35 vs 30.98±1.75 pg/mL, P<0.0001; and 53.54±3.22 vs 30.08±3.06 pg/mL, P<0.0001, respectively). Statistically significant increases (P<0.0001) were detected in RIs of right and left kidneys in PKD cats (0.72±0.01 and 0.71±0.008, respectively) compared with healthy ones (0.59±0.008 and 0.60±0.008). The statistical analysis showed a strong direct correlation between RI changes and the right or left kidney OVC (P<0.001), showing the correlation between RI increase and renal disease progression.

Conclusion: An increase in renin-angiotensin-aldosterone activity and RI in Persian cats diagnosed with PKD can be valuable diagnostic tools for their renal disease progression. However, our results showed that the systemic blood pressure is maintained and stays in its normal range.

Keywords: Aldosterone, Angiotensin II (ANG II), Persian cat, Polycystic kidney disease (PKD), Resistive index, Renin

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Introduction

Polycystic kidney disease (PKD) is a prevalent genetic disorder in felines, affecting Persian cats and outcrossed breeds. The condition is characterized by the formation of cysts in the kidneys, liver, and occasionally the pancreas. According to a previous report, PKD is responsible for 10% of chronic kidney failure in cats and has been reported with a prevalence of 37%-49% in long-hair cats, including Persian cats associated with *PKD-1* gene mutation (Vidiastuti et al., 2020). As seen in pathophysiology, the kidney tubules become cyst-like in PKD, which results in the gradual destruction of the renal parenchyma and renal failure (Bosje et al., 1998). In humans, during the manifestation and progression of PKD, increased blood pressure is found to complicate the clinical condition and lead to end-stage renal disease (ESRD) (Pedersen et al., 2003). Also, an increase in blood pressure seems to be related to the rate of renal cystic involvement in the autosomal dominant PKD and also the increase in the activity of the renin-angiotensin-aldosterone system (RAAS), caused by hypoperfusion and renal ischemia due to the development of renal cysts (Pedersen et al., 2003). An increase in blood pressure was also reported to be associated with overall renomegaly as a result of a higher number of cysts. Due to the increase in blood pressure, cardiac blood perfusion increases, and subsequently, hypertrophy of the left ventricle will be seen (Philips et al., 2007; Chapman et al., 1991).

In feline physiology, RAAS is pivotal in modulating arterial blood pressure. A surge in angiotensin II (ANG II) levels post-renal injury could theoretically affect local and systemic hemodynamics (Lourenço et al., 2022). However, empirical studies demonstrate that exogenous aldosterone administration in rats increases blood pressure and consequent renal pathology (Lourenço et al., 2022). Many nephropathic alterations in rodent models are postulated to be induced and exacerbated by aldosterone re-administration, irrespective of the pharmacological attenuation of ANG II activity (Navar et al., 1994).

Furthermore, adrenalectomy after partial nephrectomy in rodent subjects has been evidenced to mitigate the ensuing hypertensive response (Navar et al., 1994). Investigations into PKD in murine models have underscored the preeminence of the RAAS in hemodynamic regulation, noting anomalously elevated levels of angiotensin I and II in both circulatory assays and immunohistochemical analyses of polycystic renal tissues (Philips et al., 2007). Moreover, there is a marked upregulation in the expression of intrarenal renin, angiotensin II, and angiotensin-converting enzyme in these pathological contexts (Philips et al., 2007).

Diagnostic imaging is also essential for evaluating renal diseases, especially for diagnosing feline PKD (Paepe et al., 2013). Radiography and excretory urography can be used in more advanced cases to estimate renal function, but their findings will not necessarily benefit patient management; thus, ultrasonography is preferential (Guerra et al., 2019). Moreover, ultrasound can evaluate renal morphology and detect renal cysts. It is a non-invasive, safe, and inexpensive method widely available in veterinary practices (Guerra et al., 2019). Cysts are anechoic to hypoechoic spheres with thin walls in ultrasonography, ranging from a few to over ten mm. In cats older than 6 months old, ultrasound's sensitivity, specificity, and reproducibility are reported to be approximately 91%-96.2%, 91%-100%, and 100% in the diagnosis of PKD, respectively (Yu et al., 2019; Schirrer et al., 2021). Also, Doppler ultrasonography measures the blood flow velocity in renal arteries during systole and diastole, enabling the calculation of the resistive index (RI) as an indicator of renal blood flow resistance (Tipisca et al., 2016). RI assessment is helpful in diagnosing feline kidney disease and detecting renal vascular damage. Elevated RI levels in cats with kidney disease are associated with unfavorable outcomes such as renal failure and cardiovascular events (Lai et al., 2018).

The current investigation sought to assess the prognostic implications of plasma concentrations of renin, ANG II, and aldosterone as indicators of disease severity in Persian cats diagnosed with PKD during its incipient phases. Furthermore, the research aimed to elucidate the potential correlation between the RI and the severity of PKD within this specific feline cohort.

Materials and Methods

Experimental animals

Fifty Persian cats (25 with PKD and 25 healthy) were included in the present study. Animal care and the practical steps were performed per the criteria of care and use of institutional animals, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran. Without considering their gender, which was referred to the Small Animal Teaching Hospital of Faculty of Veterinary Medicine of University of Tehran from December 2021 to November 2022, Persian cats older than 6 months old were all considered potential participants in the present study.

Study design

After general examination, we performed auscultation, electrocardiogram (ECG), and echocardiography to check the cardiac condition. Complete blood count and biochemistry tests were done. Additionally, thyroid factors (T3, T4, and TSH), urinary tract infections (checked with urinalysis and culture), and urine protein to creatinine ratio were evaluated. If the Persian cats were normal in all the mentioned criteria, then they underwent fast screening abdominal ultrasonography to check whether they have polycystic or healthy kidneys. Finally, 25 PKD and 25 healthy cats were included in the study for further investigations, such as systolic blood pressure measurement, hormone analysis, and abdominal ultrasonography.

Systolic blood pressure measurement

A non-invasive cuff using an integrated sensor (SunTech Vet25, USA) measured blood pressure. An inflatable 2.5-cm cuff was tied on the animal's left clipped antebrachium, and blood pressure was measured. Blood pressure was calculated 5 times a row, and the average record was submitted. Blood pressure was recorded before any other intervention interfering with blood pressure, including blood collection, which was done for hormone analysis (Henik et al., 2005).

Blood collection and hormone analysis

After the blood pressure evaluation, blood was collected by superficial veins to reduce stress in the animal. The enzyme immunoassay (EIA) Kit was used to determine plasma renin activity (PRA) (ELH-Renin; RayBiotech; UK), ANG II (EIA-ANG II; Raybiotech; UK), and aldosterone (EU2580; Fine Test; China) levels. Blood samples were collected in prechilled tubes containing EDTA to measure these factors and stored on the ice at 4 ˚C. The samples were centrifuged at 3000×g for 20 min at 4 ˚C and kept at -80 ˚C until extraction. Within 4 months after blood sampling, the hormonal determination was performed in one ELISA run (Ward et al., 2022).

Kidney ultrasonography

The cat's abdominal hair was clipped, and after environmental adaptation (10 minutes), they were physically restrained in lateral recumbency on a table. Then, the skin was cleaned with alcohol. An expert veterinary radiologist performed an ultrasound using a high-resolution ultrasound device (Phillips Affinity 70 g, Netherlands) with a 5-12 MHz multi-frequency linear transducer. Each kidney's volume in PKD and healthy Persian cats was measured. The diameter (d) of each cyst was measured, and the volume of each cyst was derived with the sphere volume formula (V=overall volumes of all cysts in each kidney were calculated. After that, the occupying percentage of each kidney was calculated based on the total volume of all cysts of the same kidney (Debruyn et al., 2012).

Color Doppler was used to visualize the intrarenal vasculature. The RI was determined for each kidney automatically based on 3 to 5 waveform averages of the interlobar arteries (Figure 1). To measure the value of RI of the renal artery branches, the interlobar artery was first identified with color Doppler ultrasound. A gate of pulsed-wave Doppler that was <1 mm was placed on the interlobar artery. The wall filter and the pulse repetition frequency were set to the lowest values to allow the best assessment of the flow shown. Resistive indices were calculated automatically from the average of 3 to 5 waves recorded for each kidney based on the following Equation (Equation 1) (Debruyn et al., 2012; Heine et al., 2007).

1:

Resistive index=(Peak systolic velocity–End-diastolic velocity)/(Peak systolic velocity)

Statistical analysis

Statistical analysis was performed using SPSS software, version 26. Descriptive data were expressed as Mean±SEM. An independent t-test was used to analyze indices in cystic and non-cystic groups. The Pearson and Spearman correlation coefficients were used to measure the correlation between variables. A $P<0.05$ was statistically considered significant.

Results

Systolic blood pressure

The mean systolic blood pressure values were 140.92±2.35 mm Hg in PKD and 138.84±2.89 mm Hg in healthy Persian cats. No statistically significant difference was detected between PKD and healthy groups ($P>0.05$) (Figure 2).

Plasma renin, angiotensin ii, and aldosterone activity

The results of hormones analysis, including plasma renin, angiotensin II, and aldosterone activity changes in

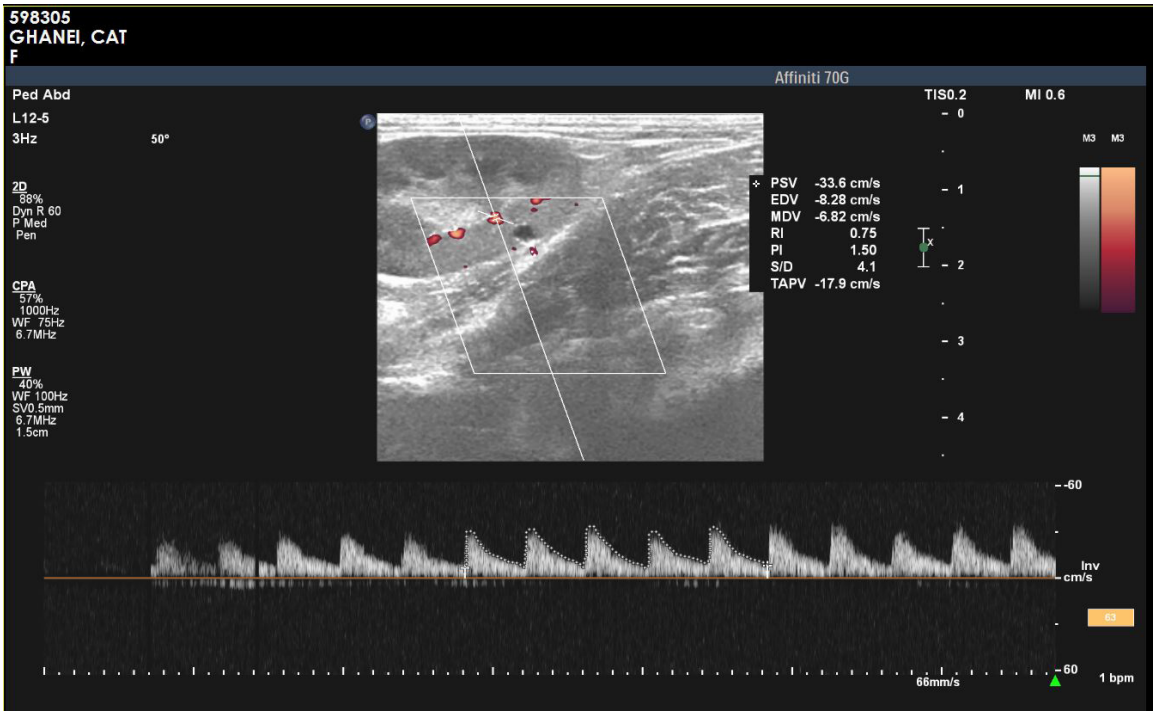

Figure 1. Color Doppler ultrasound image and measurement of the RI of an interlobar artery in a Persian cat with polycystic kidney disease

PKD and healthy Persian cats, are shown in Figure 3. Renin, angiotensin II, and aldosterone showed a statistically significant increase in the PKD group compared to the healthy group ($P<0.01$, $P<0.0001$, and $P<0.0001$, respectively).

Resistive indices analysis

The mean values for RI in the healthy and PKD cats were 0.59±0.008 and 0.71±0.01, respectively. The RI of the right and left kidneys were compared separately in the PKD and healthy groups and showed statistically significant differences (Figure 4). As shown in Figure 4, the amount of RI in the PKD group showed a significant increase in both sides of the kidneys compared to the healthy group ($P<0.0001$).

Using the Pearson correlation coefficient, an incomplete, direct, strong, and significant correlation was observed between the percentage of right and left kidney occupation with cysts and right and left kidney RI ($R=0.872$, $P<0.001$ and $R=0.858$, $P<0.001$, respectively) (Figure 5).

Discussion

The present investigation has provided insights into the potential utility of specific markers for identifying subclinical renal damage and progression in Persian cats' PKD patients. Specifically, our data demonstrated a significant elevation in renin, angiotensin II, and aldosterone levels in Persian cats with PKD, while no significant increase in mean blood pressure was observed compared to controls. The ultrasound Doppler examination in PKD Persian cats revealed significantly higher RI values (0.71±0.01) compared to healthy Persian cats (0.59±0.008). Also, this is the first reported instance of a positive correlation between RI values and the percentage of cyst occupancy in both the right and left kidneys. This study found no significant correlation between RI values and systemic blood pressure, renin, angiotensin II, and aldosterone levels in the Persian cats' participants.

Recently, there has been increasing focus on markers of subclinical renal damage. These markers can provide vital information for the early detection and management of kidney disease, especially concerning assessing the risk of cardiovascular and renal failure (Kuo et al., 2019). In human studies, endothelial dysfunction, inflammation, and insulin resistance have emerged as the most commonly encountered risk factors in the pathogenesis of cardiovascular disease in patients with PKD (Miller et al., 1999). Notably, these risk factors have been observed in the early stages of the disease, emphasizing the importance of early detection and intervention to mitigate the development of cardiovascular complications. The most significant finding from these studies is the early appear-

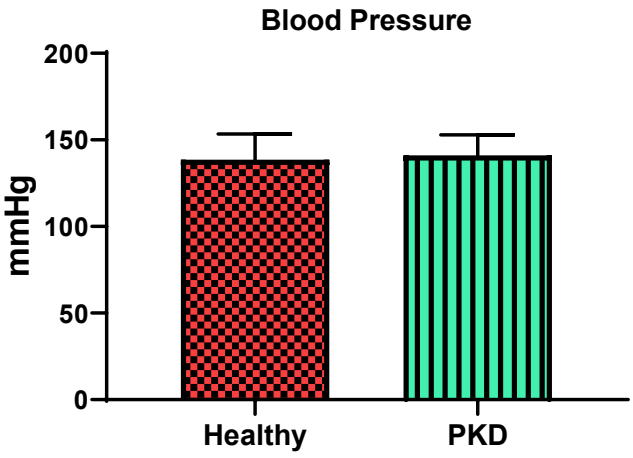

Figure 2. Systolic blood pressure in the PKD and healthy groups

Notes: Data are shown as the Mean±SD (n=25).

ance of inflammatory indexes, endothelial dysfunction, and atherosclerotic and metabolic markers, which are associated with a reduction in parameters of exercise tolerance and metabolic response indexes in PKD patients compared to healthy controls (Kuo et al., 2019). These observations highlight the need for improved risk stratification and management strategies in PKD patients to prevent the progression of cardiovascular disease and improve overall health outcomes, which may be translated into veterinary medicine as well. Accord-

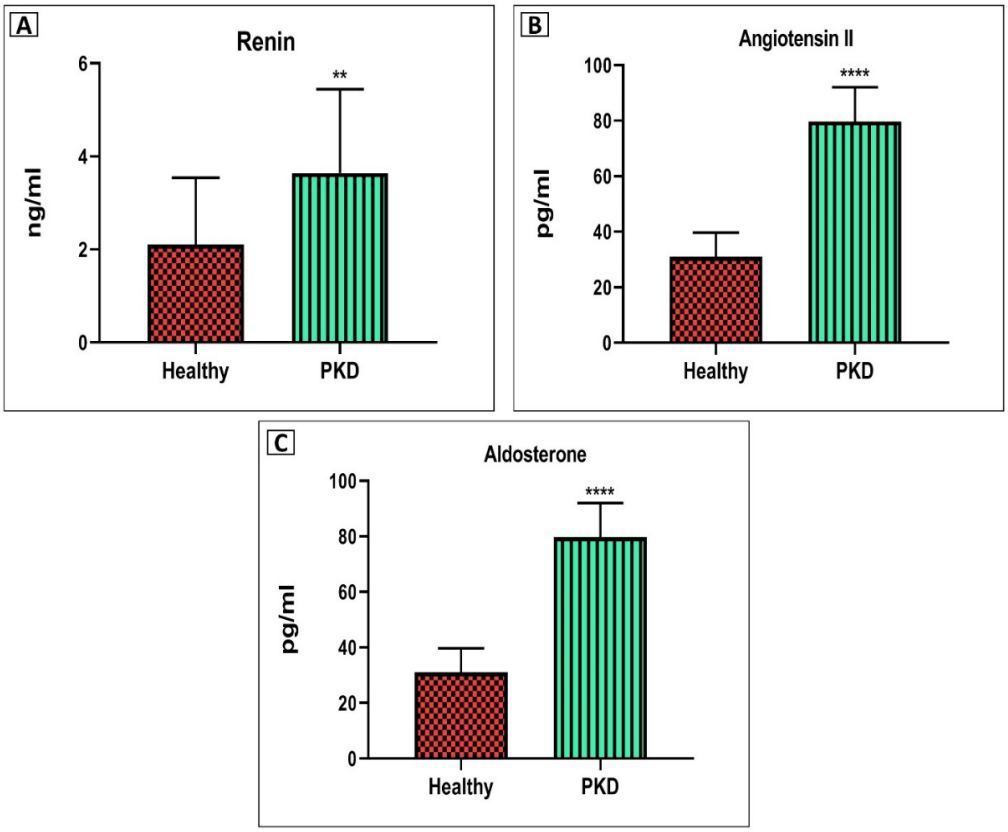

Figure 3. Renin (A), ANG II (B), and aldosterone (C) activity in the PKD and healthy groups

Notes: Data are shown as the Mean±SD, n=25. The mean values with asterisks are significantly different (P≤0.05)

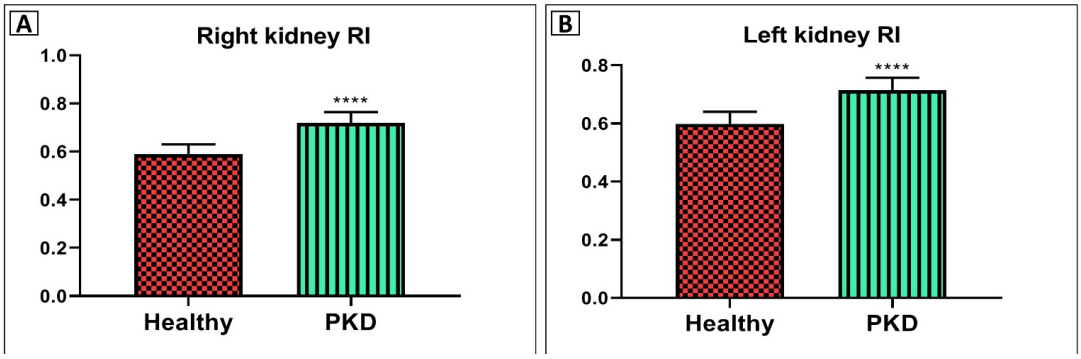

Figure 4. Resistive indices in the PKD and healthy groups

Notes: Data are shown as the Mean±SD (n=25). The mean values with asterisks are significantly different (P≤0.05).

ingly, the primary objective of this study was to bridge the gap in knowledge regarding the impact of PKD on systemic blood pressure in Persian cats. By conducting this research, we aimed to address the existing lack of information in this area and better understand the relationship between PKD and blood pressure regulation in these Persian cats.

The literature on potential prognostic markers in Persian cats with PKD is limited. In 1999, Miller and colleagues conducted a study that failed to demonstrate any significant differences in baseline blood pressure, heart rate, motor activity, RAAS status, and renal function between cats with PKD and control subjects. Moreover, PKD-afflicted cats did not observe hypertension (Miller et al., 1999). Five years later, Pedersen et al. (2003) conducted a follow-up study on 21 cats, which showed that patients with PKD had elevated mean arterial pressure and often had high angiotensin/renin ratios compared to the control group. However, no significant differences were found in serum aldosterone and PRA between the cats with PKD and the control cats. Our current study corroborates the previous findings that PKD cats do not exhibit a significant increase in systolic blood pressure. However, we observed a significant increase in the levels of major RAAS components in Persian cats with PKD. These inconsistencies in results may be attributed to variations in age, sex, breed, and blood pressure device among the studies.

Additionally, it is plausible that the disease was not severe enough to exhibit changes in the investigated indices. These observations suggest that heightened RAAS components or systolic blood pressure levels may not necessarily indicate systemic hypertension. As related studies have suggested, the relationship between serum concentrations of RAAS components and the development of systemic hypertension is multifaceted and not strictly linear, owing to counter-regulatory mechanisms, paracrine RAAS functions, and receptor dynamics. Various extraneous factors, such as dietary sodium intake, sympathetic nervous system activity, and compensatory renal responses, further modulate the physiological impact of RAAS components. Thereby underscoring the

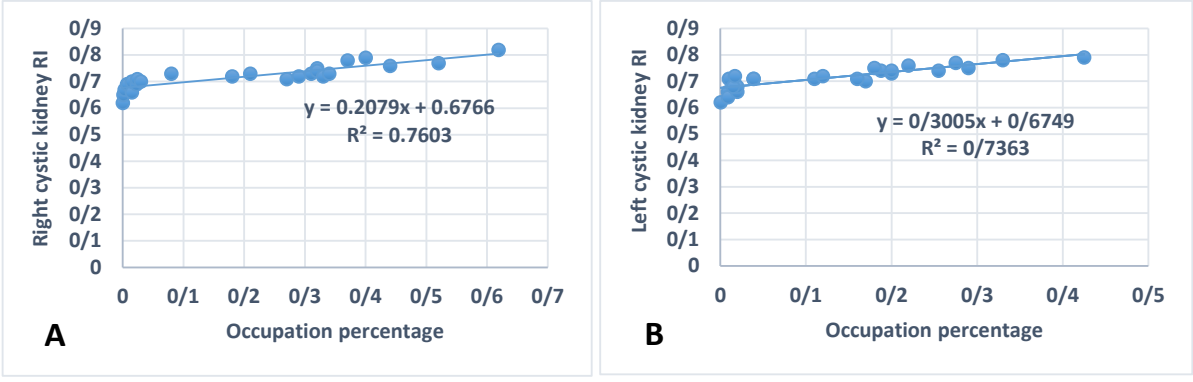

Figure 5. Scatter plot presenting the correlation between RI in right and left kidney and kidney occupation percentage with cysts in the PKD group

need for alternative markers to assess cardiovascular and mortality risk in PKD patients.

In recent scientific inquiries, RI has been employed as a metric to evaluate renal function in felines. Notably, while there is an accumulating body of research on RI values in cats diagnosed with renal diseases, specific investigations into PKD in the feline domain, with a particular emphasis on Persian cats, appear sparse. A seminal study in 2007 by Novellas et al. (2007) delved into the assessment of RI in a cohort of 10 healthy cats, arriving at a mean RI value of 0.62±0.04. Advancing the field, a subsequent study by Carvalho et al. (2011), more specialized in its approach, ascertained a mean RI of 0.52±0.06 in a sample of 25 healthy Persian cats. Crucially, a consistent finding spanning these studies is the absence of any significant variation in RI values between the right and left renal structures, a consistency that resonates with observations from our study.

In further attempts, Novellas et al. (2010) discerned a comparative RI analysis involving 20 healthy cats and 20 cats diagnosed with various renal disorders, of which 15 had chronic kidney disease (CKD), and 5 had PKD. This study elucidated that cats with renal pathologies had a discernibly elevated RI of 0.72±0.10, in contrast to the 0.62±0.04 value in their healthy counterparts. Substantiating these findings, Tipisca et al. (2016) delineated that cats suffering from CKD and acute kidney injury (AKI) exhibited significantly amplified RI values of 0.73±0.12 and 0.72±0.08, respectively, compared to healthy subjects.

In a pivotal 2018 research endeavor, Matos et al. (2018) posited an RI threshold of 0.639 as a preliminary indicator for CKD diagnosis in felines, applicable to both kidneys. Intriguingly, this benchmark closely aligns with our PKD cat study, where the lower RI limit for the PKD cohort was 0.62. Moreover, it is noteworthy that our research is the pioneering study to establish a substantial association between RI values and the extent of renal cyst occupancy. Such findings insinuate the potential of the RI as a non-invasive metric to gauge renal parenchymal damage and cyst-induced ischemia, thus underscoring its promise as a prognostic instrument.

Nonetheless, it is imperative to recognize the inherent limitations of our investigation. Constrained by a modest sample size and an absence of age and gender data, the robustness of our conclusions and the capacity to monitor disease evolution may be compromised. Future endeavors should contemplate more expansive and varied sample sizes, coupled with longitudinal methodologies, to corroborate and refine our insights. There remains a pressing exigency to explore alternative biomarkers that could facilitate the timely identification and therapeutic management of PKD in Persian cats, thereby enabling a more holistic understanding of disease trajectories and informing the conceptualization of enhanced therapeutic interventions.

Subsequently, studies focused on the possible correlation between RI values and kidney diseases. In 2010, Novellas et al. (2010) compared RI values between 20 healthy cats and 20 cats with different renal diseases, which concluded that 15 cats with CKD and 5 cats with PKD diagnosis. Their findings indicated that healthy cats exhibited a mean RI of 0.62±0.04, whereas the group with renal anomalies showed a higher value of 0.72±0.10. This finding emphasized that cats with renal anomalies tend to have an elevated RI compared to their healthy peers.

Another study by Tipisca et al. (2016) confirmed the previous study's findings, reporting that RI was significantly higher in cats with CKD (0.73±0.12) and AKI (0.72±0.08) compared to healthy cats. Finally, Matos et al. (2018) established an acceptable cut-off for the RI value of 0.639 for a preliminary diagnosis of CKD for both kidneys. Interestingly, this value is almost valid for our study on PKD cats, where the minimum RI value in the PKD group was 0.62, and none of the RI values from healthy cats exceeded this value except in a small number of animals. Moreover, our study is the first to demonstrate a significant correlation between RI values and cyst occupancy. This finding suggests that RI could be utilized as a non-invasive measure of renal parenchymal damage and ischemia induced by the cysts, thereby serving as a novel prognostic tool. Despite these promising findings, it is important to acknowledge the limitations of this study. The study was limited by its relatively small sample size and the lack of age and sexuality data, which may have affected the robustness of the findings and the ability to track disease progression over time. In light of these limitations, future research should consider larger, diverse cohorts and longitudinal study designs to validate these findings. There is also a need to explore the potential of other biomarkers for early detection and management of PKD in Persian cats. This could provide more comprehensive insights into the disease progression and help develop more effective treatment strategies.

Conclusion

Our results suggest that RI, OVC, and RAAS components are promising candidates for risk stratification and

monitoring of kidney injury in this condition. Our data demonstrated a significant elevation in renin, angiotensin II, and aldosterone levels in Persian cats with PKD, while no significant increase in mean blood pressure was observed compared to controls. The ultrasound Doppler examination in PKD Persian cats revealed significantly higher RI values than healthy Persian cats. Also, this is the first reported instance of a positive correlation between RI values and the percentage of cyst occupancy in both the right and left kidney. These markers will help inform therapeutic strategies to slow disease progression and improve renal outcomes in Persian cats with PKD.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran (Code: 76/268178).

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Authors' contributions

Conceptualization and study design: Yasamin Vali; Experiments and writing: Mahbod Ghorbani Shemirani; Data analysis: Mahbod Ghorbani Shemirani, Darioush Shirani, Shahram Jamshidi, Seyed Mahdi Nassiri, Majid Masoudifard, Maryam Mahdipour, and Yasamin Vali; Final approval: All authors.

Conflict of interest

The authors declared no conflict of interest.

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