The Effect of Hypervolemia Control on Proteinuria in Kidney Disease: A Prospective Interventional Study

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ABSTRACT

Objective: Proteinuria reduction has been extensively studied in patients with chronic kidney disease. However, the effects of diuretic treatment on proteinuria are less well-documented. This study aimed to evaluate the effects of strict volume control, achieved through diuretic therapy, on proteinuria among patients with proteinuric kidney disease and concurrent hypervolemia, as measured by bioimpedance analysis (BIA).

Materials and Methods: This prospective study included patients with proteinuria as indicated by spot urine analysis, an overhydration (OH) value of >0 in BIA, and a treatment plan that included diuretics. The follow-up period extended from the initiation of diuretic therapy, prompted by hypervolemic status, to the achievement of normovolemia in each patient.

Results: We evaluated 46 hypervolemic patients, of which 25 (54.3%) were men and 39 (64%) were women, with a mean age of 56.85±14.43 years (range 20 to 86 years). The median follow-up period was 12 days (range 5–90 days). After diuretic treatment, there was a statistically significant decrease in both systolic and diastolic blood pressure (p<0.001 for both). Initially, protein excretion in spot urine averaged 6.3 g (range 2.6–10.4 g); following volume control, proteinuria level decreased significantly to 1.5 g (range 0.6–2.9 g) (p<0.001). Additionally, regression analysis indicated a statistically significant association between the decrease in extracellular water and the decline in proteinuria levels (p=0.035).

Conclusion: Our findings demonstrate that volume control, achieved through diuretic treatment, is associated with reductions in both proteinuria and blood pressure.

Keywords: Proteinuria, kidney disease, fluid status, diuretic, bioimpedance analysis.

INTRODUCTION

It is widely recognized that patients with nephrotic-range proteinuria experience a rapid decline in kidney function. Proteinuria not only independently increases glomerular damage but also exacerbates fibrosis by inducing inflammation in the tubules.¹ Furthermore, proteinuria has been shown to elevate cardiovascular mortality rates.²
Hypertension exacerbates proteinuria through its adverse effects on the glomeruli, and antihypertensive treatment plays a crucial role in retarding nephron damage by reducing intraglomerular pressure in patients with chronic kidney disease (CKD). Consequently, blood pressure regulation is integral to the treatment of proteinuria. However, the impact of antihypertensive drugs on proteinuria varies by drug class. Renin-angiotensin-aldosterone system (RAAS) inhibitors have proven effective in reducing proteinuria and slowing the progression of CKD. Additionally, non-dihydropyridine calcium channel blockers consistently reduce albuminuria and help maintain kidney function.

The increased intraglomerular pressure and flow due to hypervolemia may perpetuate endothelial dysfunction, thereby exacerbating proteinuria and the progression of associated kidney disease. Consequently, we consider the likelihood that volume control achieved through diuretics contributes to the management of kidney disease by reducing volume load, lowering intraglomerular pressure, and decreasing proteinuria. Nonetheless, the relevance of volume control using diuretics among hypervolemic patients with proteinuric kidney disease remains understudied.

While the efficacy of RAAS inhibition in reducing proteinuria has been extensively studied, the effects of diuretic treatment on proteinuria have received less attention. Some studies have indicated that long-term oral diuretic therapy in CKD is associated with effective blood pressure control and a significant reduction in proteinuria. However, since none of these studies objectively assessed the volume status, they provided no data on the efficacy of treatment in maintaining volume control, which is a primary goal of diuretic therapy. This study aimed to evaluate the impact of diuretic therapy on proteinuria, body fluid balance, and blood pressure in patients with proteinuric kidney disease and concomitant hypervolemia, as measured by bioimpedance analysis (BIA).

**MATERIALS AND METHODS**

This prospective study was conducted in the nephrology and internal medicine clinics at a tertiary care hospital. It was carried out in compliance with the ethical principles of the Helsinki Declaration and was approved by the Ethics Committee of Erciyes University Faculty of Medicine (approval number: 2017/371). Written informed consent was obtained from each participant.

Forty-six patients were included in the study. Eligible patients had proteinuria as indicated by spot urine analysis with protein levels of >0.5 g/g, an overhydration (OH) value of >0 on BIA, and a treatment plan that included diuretics. Exclusion criteria included being on a routine hemodialysis program, unwillingness to sign the informed consent form, and the presence of any malignancy, myocardial infarction, or cerebrovascular event in the last six months, congestive heart failure, hepatic disease, estimated glomerular filtration rate (eGFR) <15 mL/min/1.73 m², and allergies to diuretics such as furosemide, hydrochlorothiazide, and spironolactone.

The diagnosis of CKD was established according to the criteria outlined in the Kidney Disease Outcomes Quality Initiative (K/DOQI) guidelines. Patients continued their routine medications, which included oral calcium channel blockers, beta-blockers, angiotensin-converting enzyme inhibitors (ACE-I), angiotensin II receptor blockers (ARB), hydrochlorothiazide, steroids, and immunosuppressive drugs, at their usual doses without any modifications. Additionally, patients were instructed to adhere to a strict salt-free diet.

**Study Design and Data Collection**

Blood pressure readings and results from blood and urine biochemical analyses were documented at the study’s onset. Measurements included blood urea nitrogen (BUN), creatinine, sodium, potassium, uric acid, total protein, albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), calcium, phosphorus, and the protein/creatinine ratio in spot urine samples.

BIA was conducted before initiating diuretic treatment to confirm the patients’ hypervolemia status and quantify the volume excess. The primary diuretic treatment began with furosemide. Patients exhibiting a slow or inadequate response to treatment, as monitored by weight, received additional treatment with hydrochlorothiazide and spironolactone, respectively. The administered doses were furosemide 40–200 mg, hydrochlorothiazide 25 mg, and spironolactone 25 mg. Due to the unavailability of standalone hydrochlorothiazide formulations in our country, hydrochlorothiazide was incorporated into the treatment regimen by substituting existing ACE-I or beta-blocker treatments with combination drugs that include hydrochlorothiazide. There were no new starts of ACE-I, ARB, or beta-blockers.

Body weight was monitored at each follow-up visit. Patients who demonstrated a reduction in body weight consistent with the achievement of normohydration, as confirmed by control BIA measurements, were considered to have completed the study. The follow-up period spanned from the initiation of diuretic therapy in response to hypervolemia to the achievement of normovolemia for each patient. Initial assessments were repeated for each patient upon completion of the study.
Body Composition Monitoring

The fluid status of patients was assessed using a Body Composition Monitor (BCM, Fresenius Medical Care, GmbH, Germany). BIA was conducted in accordance with the manufacturer’s instructions, and measurements were taken in a supine position. Electrodes were placed on the non-dominant hand and foot on the same side of the body. The BCM device connection was established using four disposable electrodes attached to the upper and lower extremities. For each participant, data on gender, height (in cm), body weight (in kg), and systolic and diastolic blood pressure (in mmHg) were recorded. The following measurements were obtained using the non-invasive bioimpedance method: overhydration value, relative hydration status (overhydration value/extracellular water), urea distribution volume, total body fluid, intercellular fluid, intracellular fluid, extracellular water (ECW), lean tissue index, adipose tissue index, and body mass index. The BCM has been extensively validated against all existing gold standard techniques in both general and dialysis populations.8 Hydration status was determined based on the OH value; patients with an OH value of ≤0 L were considered normovolemic (negatively hydrated), while those with an OH value of >0 L were identified as hypervolemic (positively hydrated). BIA measurements were performed twice during the study: initially at enrollment prior to the start of diuretic therapy, and again upon achieving normohydration, as recorded at the end of the study.

Statistical Analysis

The normality and homogeneity of the data were evaluated using the Shapiro-Wilk test and Levene’s test, respectively. All categorical data were analyzed using either the Fisher’s exact test or the Chi-squared test. Data with non-normal distributions were compared using the Mann-Whitney U test, while data with normal distributions were compared between groups using the Student’s t-test. A paired sample t-test was employed to compare normally distributed data, while the Wilcoxon test was used for data with non-normal distributions between the pre-treatment and post-treatment measurements in patients. Pearson or Spearman’s correlation analyses were performed to ascertain the relationships among proteinuria, OH, ECW, and both systolic and diastolic blood pressures, depending on the distribution of the data. Additionally, stepwise linear regression analysis was conducted to identify factors influencing the percentage change in proteinuria levels before and after treatment. Data were analyzed using the Statistical Package for the Social Sciences (SPSS, version 15.0; SPSS, Chicago, IL, USA). A p-value <0.05 was considered statistically significant.

RESULTS

Of the patients included in our study, 25 (54.3%) were male, and 21 (45.7%) were female. The mean age was 56.85±14.43 years, ranging from 20 to 86 years. The mean body mass index was 31.9±7.55 kg/m². The median follow-up period was 12 days, with a minimum of 5 days and a maximum of 90 days. The primary etiological causes of kidney disease in our patients were diabetes mellitus in 8 (17.4%) patients, hypertension in 6 (13%) patients, combined diabetes mellitus and hypertension in 18 (39.1%) patients, glomerular diseases in 13 (28.3%) patients, and amyloidosis due to familial Mediterranean fever in 1 (2.2%) patient. Among the patients with kidney disease attributed to glomerular diseases, 2 (4.4%) had focal segmental glomerulosclerosis, 6 (13%) had membranous glomerulonephritis, 3 (6.6%) had membranoproliferative Glomerulonephritis, and 2 (4.4%) had minimal change disease.

Regarding medication prior to study enrollment, 22 (47.8%) patients were on ACE inhibitors, 4 (8.7%) patients were on ARBs, 15 (32.6%) patients were on thiazide diuretics, 7 (15.2%) patients were on calcium channel blockers, and 16 (34.8%) patients were on beta blockers. New diuretic medications were introduced during the study period; furosemide alone was added to 35 (76.1%) patients, while combined diuretic treatments were prescribed for the remaining patients. In 7 (15.2%) patients, furosemide and hydrochlorothiazide were newly added, and in 4 (8.7%) patients, furosemide, spironolactone, and hydrochlorothiazide were newly added.

The mean systolic blood pressure (SBP) of the patients was 132.61±25.07 mmHg, and their diastolic blood pressure (DBP) was 78.48±12.10 mmHg before treatment. After diuretic treatment, there was a statistically significant decrease in both SBP and DBP (p<0.001 for both). Protein excretion in spot urine samples was 6.3 g (range: 2.6–10.4) before treatment. After implementing volume control, proteinuria levels significantly decreased to 1.5 g (range: 0.6–2.9) (p<0.001). To determine the effect of diuretics on proteinuria independently of other factors, we conducted an analysis excluding patients with glomerular disease. This allowed for a more accurate assessment of diuretics’ impact on our results. We observed a significant decrease in proteinuria levels following volume control, from 6.3 g to 2.1 g, after excluding 13 patients with glomerular disease from the analysis (p<0.001). Baseline proteinuria levels in patients on ACE-I or ARBs were not significantly different between groups (4.8 g vs. 8.7 g; p=0.050). Similarly, proteinuria levels at the end of follow-up did not significantly differ between users and non-users of ACE-I or ARBs (1.5 g s. 2.2 g; p=0.199). Pre-treatment body composition assessments were performed using the BIA method. The mean OH value was 3.5 L (range: 1.9–5.2). After treatment, this value decreased to 0.6 L.
Additionally, there was a statistically significant reduction in ECW from 20.45±4.51 L to 16.61±3.46 L (p<0.001). Table 1 summarizes the changes in other laboratory parameters before and after treatment.

Patients were classified according to eGFR at baseline: 18 (39.1%) were in stage 1, 9 (19.6%) in stage 2, 4 (8.7%) in stage 3a, 8 (17.4%) in stage 3b, and 7 (15.2%) in stage 4; none were in stage 5. The effects of diuretic treatment on proteinuria levels, when evaluated by CKD stages, showed a significant decline across all stages. Diuretic treatment resulted in a more pronounced decline in proteinuria among patients with early-stage CKD (stages 1 and 2) compared to those in other stages (Fig. 1). After treatment, 30 patients exhibited a decrease in eGFR, while 16 showed an increase. Proteinuria levels decreased significantly compared with pretreatment values in both patient groups, those with increased eGFR and those with decreased eGFR (p<0.001 and p=0.001, respectively).

At the start of the study, prior to diuretic treatment, a positive correlation was observed between the OH value and proteinuria levels (r=0.418, p=0.001). When evaluating percentage changes in proteinuria, blood pressure, and BIA data (OH and ECW) before and after volume load reduction, a statistically significant positive correlation was noted between proteinuria levels and both OH and ECW (r=0.360 and r=0.477, respectively).

Table 1. Comparison of pre-treatment and post-treatment values for blood pressure, bioimpedance analysis (BIA) parameters, and laboratory parameters in patients

<table>
<thead>
<tr>
<th>Variables</th>
<th>Pre-treatment</th>
<th>Post-treatment</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>132.61±25.07</td>
<td>113.91±14.67</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>78.48±12.10</td>
<td>66.52±9.24</td>
<td>&lt;0.001</td>
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<tr>
<td>eGFR (CKD-EPI, mL/dk/1.73 m²)</td>
<td>71.72±35.09</td>
<td>69.47±34.1</td>
<td>0.171</td>
</tr>
<tr>
<td>Urinary protein excretion (g/g)</td>
<td>6.3 (2.6–10.4)</td>
<td>1.5 (0.6–2.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sodium (mEq/L)</td>
<td>138.39±3.20</td>
<td>137.5±3.42</td>
<td>0.223</td>
</tr>
<tr>
<td>Potassium (mEq/L)</td>
<td>4.64±0.50</td>
<td>4.40±0.59</td>
<td>0.002</td>
</tr>
<tr>
<td>Total protein (g/dL)</td>
<td>5.78±0.99</td>
<td>6.30±0.98</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>2.81±0.83</td>
<td>3.20±0.86</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Uric Acid (mg/dL)</td>
<td>6.76±1.77</td>
<td>7.75±2.13</td>
<td>0.010</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>22.1±8.44</td>
<td>20.50±7.54</td>
<td>0.467</td>
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<tr>
<td>ALT (U/L)</td>
<td>16.0 (12–23.2)</td>
<td>12.5 (10.2–20.5)</td>
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<td>Calcium (mg/dL)</td>
<td>8.55±0.73</td>
<td>8.77±0.73</td>
<td>0.069</td>
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<tr>
<td>Phosphorus (mg/dL)</td>
<td>3.98±0.73</td>
<td>4.20±0.94</td>
<td>0.062</td>
</tr>
<tr>
<td>Bioimpedance analysis parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OH (L)</td>
<td>3.5 (1.9–5.2)</td>
<td>0.6 (-0.1–1.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>OH/ECW (relative hydration status)</td>
<td>17.3 (10.3–23.4)</td>
<td>3.5 (-0.9–7.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>84.09±18.30</td>
<td>79.66±17.50</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ECW (L)</td>
<td>20.45±4.51</td>
<td>16.61±3.46</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SD or median (including the lower and upper quartiles). SD: Standard deviation; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ECW: Extracellular water; eGFR: Estimated glomerular filtration rate; OH: Overhydration value.
respectively). However, Table 2 indicates no significant correlation between changes in SBP and DBP and changes in OH, ECW, and proteinuria levels.

Table 3 summarizes the factors affecting percentage change in proteinuria levels before and after treatment, as evaluated by stepwise linear regression analysis. The coefficient of determination (R-Squared) of the model was 0.239, and the root mean squared error (RMSE) of the residuals was 4.176 g. A statistically significant relationship was found between the decrease in ECW and proteinuria levels (p=0.035). No significant relationship was observed between the changes in SBP, DBP, OH, and eGFR levels and the changes in proteinuria levels.

DISCUSSION
Proteinuria is an independent risk factor for the progression of kidney disease.\textsuperscript{9,10} and is associated with an increased risk of cardiovascular disease and mortality.\textsuperscript{11-13} It can cause nephrogenic damage, including interstitial damage and fibrosis. This damage results not only from glomerular injury but also from the increased inflammation and fibrogenic effects due to protein-tubular cell interactions in the tubules. Thus, reducing proteinuria in proteinuric nephropathies is a critical therapeutic goal.\textsuperscript{12} While literature suggests that diuretic treatment can reduce proteinuria in CKD patients, none of these studies have evaluated the impact of diuretic treatment on changes in volume status over time by measuring patients' volume levels. Given that the primary aim of diuretic treatment is to achieve normovolemia, this study is the first to objectively demonstrate the relationship between diuretic treatment, volume status, and proteinuria, based on BIA measurements. Our findings show a significant reduction in proteinuria levels following strict volume control in hypervolemic patients receiving diuretic therapy, without changes in ACE-I, ARB, or immunosuppressive therapy usage. Proteinuria levels significantly decreased as the volume load was reduced. Additionally, there was a statistically significant positive correlation between proteinuria levels and both OH and ECW. Regarding blood pressure, both SBP and DBP levels significantly decreased. However, there was no statistically significant decrease in eGFR levels with diuretic therapy. These results underscore that proteinuria can be effectively reduced in patients with hypervolemic proteinuric kidney disease through strict volume control, and that diuretics are beneficial in this context.
In a related study by Hoshino et al., involving patients with diabetic kidney disease and accompanying edema, significant improvements in blood pressure and proteinuria levels were observed with thiazide and loop diuretic therapy, without adverse effects on GFR. One-year diuretic treatment was reported to be associated with a 65% reduction from baseline in proteinuria levels by Hoshino et al. In contrast, our study achieved a 77% reduction. This discrepancy may be attributed to the prolonged diuretic treatment until normovolemia was achieved, as determined by BIA in our study, and the adjustment of diuretic doses based on volume status. Consequently, effective volume control achieved through BIA measurement appears to further reduce proteinuria levels in our study.

Hayashi et al. indicated that adding diuretic treatment to the regimen of hypertensive CKD patients significantly lowered proteinuria and enhanced blood pressure control. Ensaut et al. found that adding a loop diuretic to ACE + ARB therapies at various doses significantly decreased urinary protein excretion. Similarly, Fujisaki et al. reported that supplementing losartan therapy with hydrochlorothiazide led to a significantly greater decline in proteinuria levels compared to patients receiving only losartan, despite similar blood pressure levels. Zamboli et al. compared RAAS inhibition and furosemide treatments in hypertensive CKD patients and reported a significantly greater reduction in proteinuria levels with furosemide treatment. Additionally, like our findings, they reported no significant decrease in eGFR levels over a 52-week period. However, the absence of BIA measurements in these studies leaves the exact changes in volume status undetermined.

Diuretics normalize blood pressure by reducing extracellular fluid volume through natriuresis. Therefore, the resultant improvement in systemic hemodynamic balance is believed to concurrently improve kidney hemodynamics, including intraglomerular pressure reduction. Moreover, diuretic treatment combined with strict volume control can enhance endothelial function, reduce inflammation, and stimulate the tubuloglomerular feedback mechanism. These improvements in glomerular parameters may explain the observed decrease in proteinuria levels under diuretic treatment.

One of the primary factors contributing to the development of hypertension in patients with CKD is the expansion of ECW due to fluid and sodium retention. Consequently, diuretics play a crucial role in managing hypervolemia and regulating blood pressure in these patients. In a study conducted by De Nicola et al. on hypertensive CKD patients, only 12% achieved blood pressure control despite using multiple antihypertensive drugs. The researchers linked the inadequate control of blood pressure to poor management of ECW, noting that most study participants either did not receive diuretic treatment (63%) or received it at inadequate doses. Abe et al. observed that adding thiazide diuretics to the antihypertensive regimen significantly lowered blood pressure in patients with stage 3–4 CKD and provided renoprotective benefits by reducing urinary protein excretion. They recommended incorporating diuretic therapy for patients with CKD who do not achieve adequate blood pressure control with maximum doses of RAAS inhibitors. Fujisaki et al. reported no significant difference in blood pressure among CKD patients treated either with ARBs alone or with combined ARB + thiazide diuretics after one year. However, the need for additional antihypertensive medication was higher in the group treated with ARBs alone. Yesiltepe et al. highlighted the importance of maintaining a negative hydration status for blood pressure control in hypertensive patients without kidney failure. In our study, we observed a significant reduction in mean SBP and DBP levels following diuretic treatment. Given the notable decrease in blood pressure levels, it is plausible to consider that the impact of diuretics on proteinuria might primarily be due to their blood pressure-lowering effects. However, our stepwise linear regression model, which exhibited a positive R-Squared value of 0.239 and an average error rate of nearly 4 g, did not show a significant relationship between changes in SBP, DBP, and proteinuria levels. The only statistically significant relationship was found between the reduction in ECW and proteinuria levels. Nonetheless, the RMSE statistic from our fitted regression model limits its utility for predictive purposes, indicating that statistical significance between ECW and proteinuria does not necessarily imply clinical relevance.

Previous studies have demonstrated that managing hypervolemia in CKD can decelerate disease progression and decrease mortality rates. Esmeray et al. reported that, after one year of follow-up, hypervolemic patients exhibited lower eGFRs, higher systolic blood pressure levels, and increased mortality rates compared to normovolemic patients. This study demonstrates, for the first time in the literature, that one of the key factors contributing to the retardation of CKD progression and reduction in mortality rate appears to be the proteinuria-reducing effect of normovolemia, as objectively evidenced in the current analysis. Moreover, our findings indicate a positive correlation between volume decrease and proteinuria reduction. The decline in ECW was the most influential factor in decreasing proteinuria in our cohort, supporting the hypothesis that correcting hypervolemia reduces proteinuria, thereby slowing the progression of kidney dysfunction and reducing mortality rates.

Reducing proteinuria levels in patients with CKD due to primary glomerulonephritis is an important issue. Immunosuppressive agents such as calcineurin inhibitors, alkylating agents, and mycophenolate mofetil are commonly
used in these patients’ treatment. Although these agents effectively reduce proteinuria, they also carry serious side effects.27,28 In our study, proteinuria levels were significantly reduced by 77.8% through strict volume control alone, without any modifications in immunosuppressive treatments in patients with glomerulonephritis.

A hypervolemic state, assessed by BIA in patients with CKD, is strongly associated with proteinuria, even in the non-nephrotic range, as indicated in Schork et al.’s29 cross-sectional study. A novel finding of our study is that proteinuria can be reduced by establishing normovolemia through diuretic therapy in a prospective design, involving two BIA measurements. This approach is also applicable to patients with CKD and glomerulonephritis. Clinically, hypervolemia in these patients often results in proteinuria and hypertension, necessitating the use of diuretics.

A major limitation of the current study is the lack of long-term follow-up data on our patients, which prevents us from assessing the sustained effects of our treatment approach. Additionally, including a larger cohort of patients, particularly those with advanced chronic renal failure, might have yielded more substantial results. Moreover, the relatively high RMSE of the fitted regression model in our study limits its applicability in clinical practice.

CONCLUSION

Our findings highlight the association between volume control achieved through diuretic treatment and reductions in proteinuria and blood pressure, without significant changes in eGFR. These results underscore the importance of maintaining normovolemia for kidney protection and reducing mortality in patients with CKD.

Ethics Committee Approval: The Erciyes University Clinical Research Ethics Committee granted approval for this study (date: 07.07.2017, number: 2017/371).

Author Contributions: Concept – ZBG, OSD, AIG; Design – ZBG, OSD, AIG; Materials – ZBG, OSD, AIG; Data Collection and/or Processing – ZBG, OSD, AIG; Analysis and/or Interpretation – ZBG, OSD, AIG; Literature Search – ZBG, OSD, AIG; Writing – ZBG, OSD, AIG; Critical Reviews – ZBG, OSD, AIG.

Conflict of Interest: The authors have no conflict of interest to declare.

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