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Original Paper

Efficacy of Probiotics Supplementation On Chronic Kidney Disease: a Systematic Review and Meta-Analysis

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Key Words
Probiotics • Chronic kidney disease • Uremic toxin • Intestinal microbiota • Randomized controlled trial

Abstract
Background/Aims: Dysbiosis of the intestinal microbiota may accelerate the progression of chronic kidney disease (CKD) by increasing the levels of urea toxins. In recent years, probiotics have been recognized to maintain the physiological balance of the intestinal microbiota. In this study, we aim to assess the therapeutic effects of probiotics on CKD patients with and without dialysis via meta-analysis. ***Methods:*** We conducted a meta-analysis of randomized controlled trials (RCTs) by searching the databases of Pubmed, EMBASE and Cochrane Library (No. CRD42018093080). Studies on probiotics for treatment of CKD adults lasting for at least 4 weeks were selected. The primary outcomes were the levels of urea toxins, and the second outcomes were the levels of interleukin (IL)-6, C-reactive protein (CRP) and hemoglobin (Hb). The risk of bias was assessed by Cochrane Collaboration' tool, and the quality of evidence was appraised with the Grading of Recommendation Assessment. Means and standard deviations were analyzed by random effects analysis. Stratified analysis was done and sensitivity analysis was performed when appropriate. ***Results:*** Totally, eight studies with 261 patients at CKD stage 3 to 5 with and without dialysis were included. We found a decrease of p-cresyl sulfate (PCS) of 3 studies with 125 subjects (P = 0.01, SMD -0.57, 95% CI, -0.99 to -0.14, I^2 = 25%) and an increase of IL-6 in 3 studies with 134 subjects (P = 0.03, 95% CI, SMD 0.37, 0.03 to 0.72, I^2 = 0%) in the probiotics groups. Analysis of serum creatinine (P = 0.47), blood urine nitrogen (P = 0.73), CRP (P = 0.55) and Hb (P = 0.49) yielded insignificant difference. ***Conclusion:*** Limited number of studies and small sample size are limitations of our study. Probiotics supplementation may reduce the levels of PCS and elevate the levels of IL-6 whereby protecting the intestinal epithelial barrier of patients with CKD.

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Introduction

Chronic kidney disease (CKD), especially end-stage renal disease (ESRD) threatens the global health and leads to various health problems like cardiovascular diseases [1]. Although medication or renal replacement therapies may delay the progression of CKD to some extent [2], more than 2 million people worldwide are diagnosed with end stage renal disease [3], which is a substantial burden for global health and economics [4].

The progression of CKD might be influenced by several factors, such as dietary intake, mental stress, medications and so forth [4]. Recent studies revealed the importance of the gut microbiota in the development and progression of CKD [5]. Dysbiosis of the intestinal microbiota increases urea toxins, such as indole-3 acetic acid, p-cresyl sulfate (PCS) and indoxyl sulfate [6], which damage the epithelial tight junctions and increase the permeability of the intestinal wall via endotoxemia and systemic inflammation [7]. As a consequence, intestinal endotoxins may go through the intestinal wall into the blood circulation, induce microinflammation in kidney and cause renal endothelial dysfunction, fibrosis, and tubular damages, which subsequently accelerates the decline of renal function [8, 9].

Probiotics supplementation has emerged as an adjuvant therapy for CKD in recent years, because probiotics cost lower and are more acceptable by patients. Researchers have investigated in many studies whether the probiotics could slow down the progression of CKD by regulating the intestinal flora alteration and by reducing the urea toxin. Even in different randomized controlled trials (RCTs), however, the therapeutic regimens of probiotics were inconsistent. Some researchers found a positive effect of probiotics on decreasing inflammation biomarkers in CKD patients [10], while others reported no remarkable changes [11]. A variety of confounding factors, such as treatment duration, diversity of strains, sample size, etc, make studies difficult to be compared directly. Thus, it is necessary to evaluate the therapeutic effects of probiotics on CKD by an evidence-based method.

In this study, we first systematically searched PubMed, EMBASE and Cochrane library for RCTs on probiotics and CKD and assessed the therapeutic effectiveness, including indicators of urea toxins, inflammation and anemia, of the probiotics supplementation compared with placebo on CKD patients. We further identified effective intervention modalities, including dosage and duration. Finally, we highlighted gaps in literature for guiding future follow-up studies in this field.

Materials and Methods

The study was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Supplementary Table S1), and the protocol was registered at International Prospective Register of Systematic Reviews (https://www.crd.york.ac.uk/PROSPERO/, No. CRD42018093080) (for all supplementary material see www.karger.com/doi/10.1159/000494677).

Searching strategy

We searched articles in three electronic databases, i.e. PubMed, EMBASE and Cochrane Library. Medical Subject Headings and entry terms of "chronic kidney disease", "end stage renal disease" and "probiotics" were combined in the searching system (Supplementary Methods Section). All of the English publications until 31 March 2018 were searched without any restriction of origins or article type. The reference list of all selected articles was independently screened by two reviewers to identify additional studies left out in the initial search.

Study selection and outcome assessment

RCTs in which the probiotics were administered for at least 4 weeks to adult CKD patients, irrespective of whether the patients had received dialysis at the baseline, were included. If one cohort was described in several articles, we chose the article with the largest sample size and/or the longest duration. Animal

studies, *in vitro* experiments, and non-RCT clinical trials, as well as publications lacking sufficient data, such as narrative reviews, case reports/series and conference abstracts, were excluded. The primary measured outcomes were changes of urea toxins. The secondary outcomes were hemoglobin (Hb), interleukin (IL)-6 and C-reactive protein (CRP). Detailed outcomes were defined as follows.

1. Primary outcomes. We pooled data of blood urea nitrogen (BUN) and serum creatinine (Scr) at the unit of mg/dL in selected studies. Negative differences in BUN and Scr represent a significant decrease in the probiotics group compared with the placebo group. The unit of PCS is μg/mL.

2. Secondary outcomes. Hb data were pooled and converted to the unit of g/dL. Data of CRP was collected with the unit of mg/dL. Negative differences in IL-6 represent a significant decrease in the probiotics group compared with the placebo group. The unit of IL-6 was pg/mL.

All articles were assessed by two reviewers independently (Linpei Jia and Hongliang Zhang). After the initial search, we first looked through the titles and abstracts to determine eligible studies to be included. We then assessed the full-texts to determine the studies to be included for meta-analysis. Discrepancies in the selection process were discussed with a third researcher (Rufu Jia) for accuracy of selection.

Data extraction

Published reports were obtained for each eligible trial, and related information was extracted by two independent reviewers (Linpei Jia and Qiang Jia) into an Excel document. Discrepancies of data extraction were discussed and solved by consensus with the help of a third reviewer (Rufu Jia). The extracted data included characteristics of study (publication year, names of authors, countries of study, study duration and withdraws of participants), information of participants (sample size, age, sex, inclusion criteria, exclusion criteria and status of dialysis), details of the probiotics supplementation (dosage, component and duration of treatment) and outcomes.

Quality assessment and summary of findings (SoF)

The risk of bias of included studies were estimated by taking into consideration the characteristics including random sequence generation, allocation concealment, blinding of patients, blinding of outcome assessment, completeness of outcome data, selective reporting and other bias by Cochrane Collaboration's tool for assessing the risk of bias [12]. Quality of evidence was appraised with the Grading of Recommendation Assessment (GRADE) method including the risk of bias, inconsistency, indirectness, imprecision, and publication bias [13] by the GRADEpro GDT 2015 to create an SoF table.

Quality assessment and GRADE were independently performed by two researchers (Jingyan Yang and Hongliang Zhang). Disagreements over the risk of bias in particular studies will be resolved by discussion, which routinely implicated a third researcher (Rufu Jia) if necessary.

Data pooling and analysis

Since all the indicators in our meta-analysis were continuous variables, means and standard deviations of each outcome were collected and analyzed by the inverse variance method in random effects analysis [14]. The mean difference was used as effect measures, while the standard mean difference was used for PCS and IL-6, because testing methods of these two outcomes were with large differences [15]. Missing means and standard deviations were input by median data, interquartile range and full range [16]. We calculated the percentage of variability among studies attributable to heterogeneity beyond chance by I^2 statistics. Further subgroup analysis of CKD patients with and without dialysis in each outcome was conducted. We performed a sensitivity analysis to assess the stability of the results as well. $P < 0.05$ was considered as statistically significant. All the statistics were done by the RevMan version 5.3 (The Nordic Cochrane Centre, Cochrane Collaboration, Copenhagen, Denmark).

Results

Eight studies were finally enrolled for data analysis

Initially, 288 publications were searched from PubMed, EMBASE and Cochrane Library. After reviewing titles, abstracts and full texts, only 9 articles of 8 studies [2, 10, 11, 17-22]

were selected for our meta-analysis (Fig. 1). A total of 261 subjects at CKD stage 3 to 5 were analyzed. Since three studies were cross-over designed [19, 21, 22], 99 subjects acted as both tests and controls. The basic characteristics of selected studies were shown in Table 1. Among all the participants, 130 patients received dialysis, among whom 91 received hemodialysis and 39 received peritoneal dialysis [10, 11, 17, 19]. Eight different probiotics were studied, and the treatment duration ranged from 1 to 6 months. The daily dose of probiotics ranged from 4 to 180 billion colony-forming unit (CFU).

Assessment of risk of bias

The κ coefficient of two reviewers was 0.899 ($P < 0.05$). The bias of included trials was assessed according to the Cochrane Collaboration's tool for assessing the risk of bias (Fig. 2 and Supplementary Table S2). Blinding of outcome assessment had the lowest low risk of 25%, while incomplete outcome data and other bias had the highest risk of 87.5% (Fig. 2a). Borges' [17] studies was graded as highest quality, while Pavan's [20] study was graded as low quality (Fig. 2b).

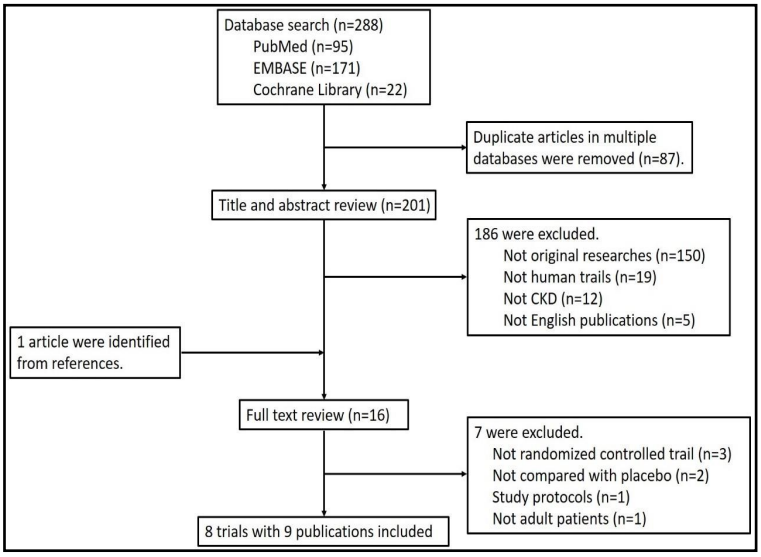

Fig. 1. Flow chart of identification of eligible studies. Initially 288 articles were searched from three databases, including 95 in PubMed, 171 in EMBASE and 22 in the Cochrane Library. Then 87 duplicates were removed. After reviewing titles and abstracts, 186 articles were excluded. At the same time, one study was identified from references of selected articles. Two independent researchers read the fulltext of the remaining 16 studies, then 7 of them were removed for the nature of non-RCTs, study protocols, pediatric studies and no-placebo trials. Finally, 9 publications of 8 trials were included for analysis in our study.

Table 1. Characteristic studies of meta-analysis. NR: not reported. The age of each study is shown as mean ± standard deviation or mean (range)

Study	Sample size	Male ratio %	Mean age, y	Stage of chronic kidney disease	Dialysis status	Component of probiotics per capsule	Dosage of probiotics	Duration of Treatment
Rangananthan 2010 [21]	46 subject/ controls (cross-over study)	67.4	55.4±12.4	3 or 4	No	A mix of L. acidophilus, B. longum, and S. thermophiles for a total of $1.5X10^{10}$ colony-forming unit (CFU).	A daily dose of 90 billion CFU	3 months
Rangananthan 2014 [19]	22 subjects/ controls (cross-over study)	83.2	54 (29-79)	5	Hemodialysis	30 billion CFU of S. thermophilus, L. acidophilus and B. longum.	A daily dose of 180 billion CFU	2 months
Guida 2014 [18]	18 subjects and 12 controls	86.7	59.5±13.1	3 or 4	No	$5X10^9$ Lactobacillus plantarum, $2X10^9$ Lactobacillus casei subsp. rhamnosus and $2X10^9$ Lactobacillus gasseri, $1X10^9$ Bifidobacterium infantis and $1X10^9$ Bifidobacterium longum, $1X10^9$ Lactobacillus acidophilus, $1X10^9$ Lactobacillus salivarius and $1X10^9$ Lactobacillus sporogenes and $5X10^9$ Streptococcus thermophilus.	A daily dose of 57 billion CFU	1 month
Wang 2015[10]	21 subjects and 18 controls	46.2	NR	5	Peritoneal dialysis	$1X10^9$ CFU B. bifidum, $1X10^9$ CFU B. catenulatum, $1X10^9$ CFU B. longum, and $1X10^9$ CFU L. plantarum.	A daily dose of 4 billion CFU	6 months
Pavan 2016 [20]	12 subjects and 12 controls	66.7	57.8±7.11	3 to 5	No	$15X10^9$ CFU Streptococcus thermophiles, $15X10^9$ CFU Lactobacillus acidophilus and $15X10^9$ CFU L. plantarum.	A daily dose of 135 billion CFU	6 months
Rossi 2016 [22]	31 subjects/ controls (cross-over study)	61	69	4 or 5	No	Lactobacillus, Bifidobacteria, and Streptococcus genera.	A daily dose of 45 billion CFU at first 3 weeks, and 90 billion CFU after 3 weeks	6 weeks
Shariaty 2017 [11]	18 subjects and 18 controls	55.6	57.8 (47-60)	5	Hemodialysis	$15×10^9$ CFU L. acidophilus, $1.5X10^9$ CFU Lactobacillus casei, $3.5X10^9$ CFU Lactobacillus rhamnosus, $0.25X10^9$ CFU Lactobacillus bulgaricus, $10X10^9$ CFU Bifidobacterium breve, $0.5X10^9$ CFU Bifidobacterium longum, and $0.15X10^9$ CFU L. plantarum.	A daily dose of 30.9 billion CFU	12 weeks
Borges 2018 [17]	16 subjects and 17 controls	63.6	51.9±9.79	5	Hemodialysis	Streptococcus thermophilus, Lactobacillus acidophilus, and Bifidobacteria longum.	A daily dose of 90 billion CFU	3 months

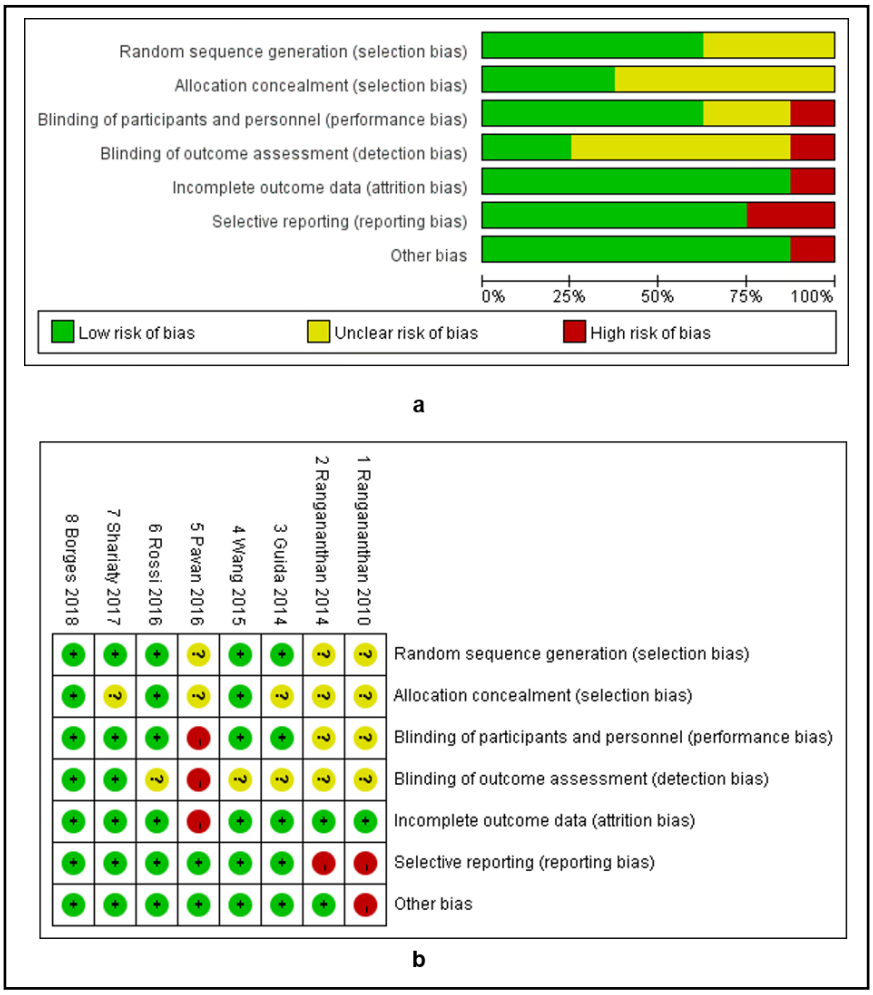

Fig. 2. Risk of bias graph and summary. In Fig. 2a, low risk of random sequence generation was 62.5%, and low risk of allocation concealment was 37.5%. Blinding of patients had the low risk of 62.5%, while blinding of outcome assessment had the low risk of 25%. Completeness of outcome data and other bias were with the highest rate of low risk of 87.5%. Selective reporting with the low risk of 75%. In Fig. 2b, color green represents low risk, while red represents high risk. The yellow circle represents unclear risk, which means that no evidence was found. Borges' study was estimated as low risks in all assessments. And Pavan's research was estimated as high risk in three assessments.

Hb and PCS were assessed as low-quality outcomes

The quality of included outcomes was shown in a SoF table (Table 2). The κ coefficient of two reviewers was 1.00 ($P < 0.05$). The assessment of evidence quality ranged from low to very low across outcomes. Scr, BUN, IL-6 and CRP were estimated as very low quality, while Hb and PCS were assessed as low qualities. For low-quality outcomes, we had limited confidence of the results. For very low-quality indicators, we had the limited credibility about the results.

The probiotics supplementation could decrease PCS of CKD patients

Five studies [10, 17, 20-22] with 126 subjects who received the probiotics supplementation and 124 subjects who received placebos reported changes of Scr. No significant changes of Scr were found between probiotics and placebo groups with mean difference of 0.08 mg/dL ($P = 0.47$, 95% CI, -0.13 to 0.28, $I^2 = 0\%$, Fig. 3a). Pavan and Rossi's studies [20, 22] had larger weight in the analysis of Scr. In the subgroup analysis of Scr, mean differences of 0.10 mg/dL for patients without dialysis ($P = 0.36$, 95% CI, -0.11 to 0.31, $I^2 = 0\%$, Fig. 3a) and -1.16 mg/dL for patients with dialysis ($P = 0.14$, 95% CI, -2.73 to 0.04, $I^2 = 0\%$, Fig. 3a) were shown. However, no significant differences of Scr were found in subgroup analyses.

Table 2. Summary of findings of probiotics for chronic kidney disease (CKD). Patient or population: patients with chronic kidney disease, Settings: hospital, Intervention: probiotics. *The basis for the assumed risk (e.g. the median control group risk across studies) is provided in footnotes. The corresponding risk (and its 95% confidence interval) is based on the assumed risk in the comparison group and the relative effect of the intervention (and its 95% CI). CI: Confidence interval. GRADE Working Group grades of evidence: High quality: Further research is very unlikely to change our confidence in the estimate of effect. Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate. Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate. Very low quality: We are very uncertain about the estimate. [1]Some concerns with blinding of patients, blinding of outcome assessment, completeness of outcome data and selective reporting. Thus, the quality of evidence was downgraded with one level. [2]Imprecise due to the small sample size (less than 300) in all researches. Thus, the evidence quality was downgraded as one level. [3]Some concerns with selected trials conducted by drug companies, thus the evidence quality was downgraded by one level. [4]Some concerns with completeness of outcome data, selective reporting and other bias. The quality of evidence was downgraded with one level. [5]Three studies were inconsistent in blood urea, thus the evidence quality was downgraded by two levels. [6]One study was inconsistent with the other two in interleukin-6, thus the quality of evidence was downgraded by one level. [7]Some concerns with blinding of patients, blinding of outcome assessment and completeness of outcome data. Thus, the quality of evidence was downgraded by one level. [8]One study was inconsistent with the other two in C-reactive protein, thus the quality of evidence was downgraded by one level

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No of Participants (studies)	Quality of the evidence (GRADE)	Comments
	Assumed risk	Corresponding risk				
	Control	Probiotics				
Serum creatinine Follow-up: 6 weeks to 6 months		The mean serum creatinine in the intervention groups was 0.08 mg/dL higher (0.13 lower to 0.28 higher)		250 (5 studies)	*- - - very low[1,2,3]	We are uncertain about the effect of probiotics on serum creatinine of CKD patients compared with the placebo.
Blood urea Follow-up: 3 to 6 months		The mean blood urea in the intervention groups was 1.38 mg/dL lower (9.26 lower to 6.5 higher)		164 (3 studies)	*- - - very low[2,3,4,5]	We are uncertain about the effect of probiotics on blood urea of CKD patients compared with the placebo.
p-cresyl sulfate Follow-up: 1 to 3 months		The mean p-cresyl sulfate in the intervention groups was 0.57 standard deviations lower (0.99 to 0.14 lower)		125 (3 studies)	**- - low[2,3]	SMD -0.57 (-0.99 to -0.14) Probiotics may slightly improve p-cresyl sulfate of CKD patients compared with the placebo.
Hemoglobin Follow-up: 3 to 6 months		The mean hemoglobin in the intervention groups was 0.21 g/dL higher (0.48 lower to 0.91 higher)		93 (3 studies)	**- - low[2,7]	Probiotics may slightly improve hemoglobin of CKD patients compared with the placebo.
Interleukin-6 Follow-up: 6 weeks to 6 months		The mean Interleukin-6 in the intervention groups was 0.37 standard deviations higher (0.03 to 0.72 higher)		134 (3 studies)	*- - - very low[2,3,6]	SMD 0.37 (0.03 to 0.72) We are uncertain about the effect of probiotics on interleukin-6 of CKD patients compared with the placebo.
C-reactive protein Follow-up: 2 to 3 months		The mean C-reactive protein in the intervention groups was 0.64 md/dL higher (3.81 lower to 5.09 higher)		106 (3 studies)	*- - - very low[2,3,8]	We are uncertain about the effect of probiotics on C-reactive protein of CKD patients compared with the placebo.

Changes of BUN were available in three studies with 164 participants including 83 in probiotics and 81 in placebo groups. The result of meta-analysis showed no significant changes of BUN between the two groups (P = 0.73). Compared with the controls, a total of 1.38 mg/dL (95% CI, -9.26 to 6.50, I^2 = 0%, Fig. 3b) BUN was decreased in the probiotics group. Wang's data weighed largest in the analysis. Similar results of BUN were found in dialysis patients (P = 0.76, 95% CI, -12.68 to 17.32, I^2 = 30%, Fig. 3b) in the subgroup analysis, since only one study was included in the non-dialysis subgroup.

Meanwhile by analyzing PCS data of 65 subjects receiving probiotics and 60 subjects receiving placebos in three studies [17, 18, 22], a significant decrease of PCS in CKD patients was shown after taking probiotics (P = 0.01, SMD -0.57, 95% CI, -0.99 to -0.14, I^2 = 25%, Fig. 3c). The same result was shown in subgroup analysis in patients without dialysis (P = 0.05, 95% CI, -1.40 to -0.01, I^2 = 56%, Fig. 3c).

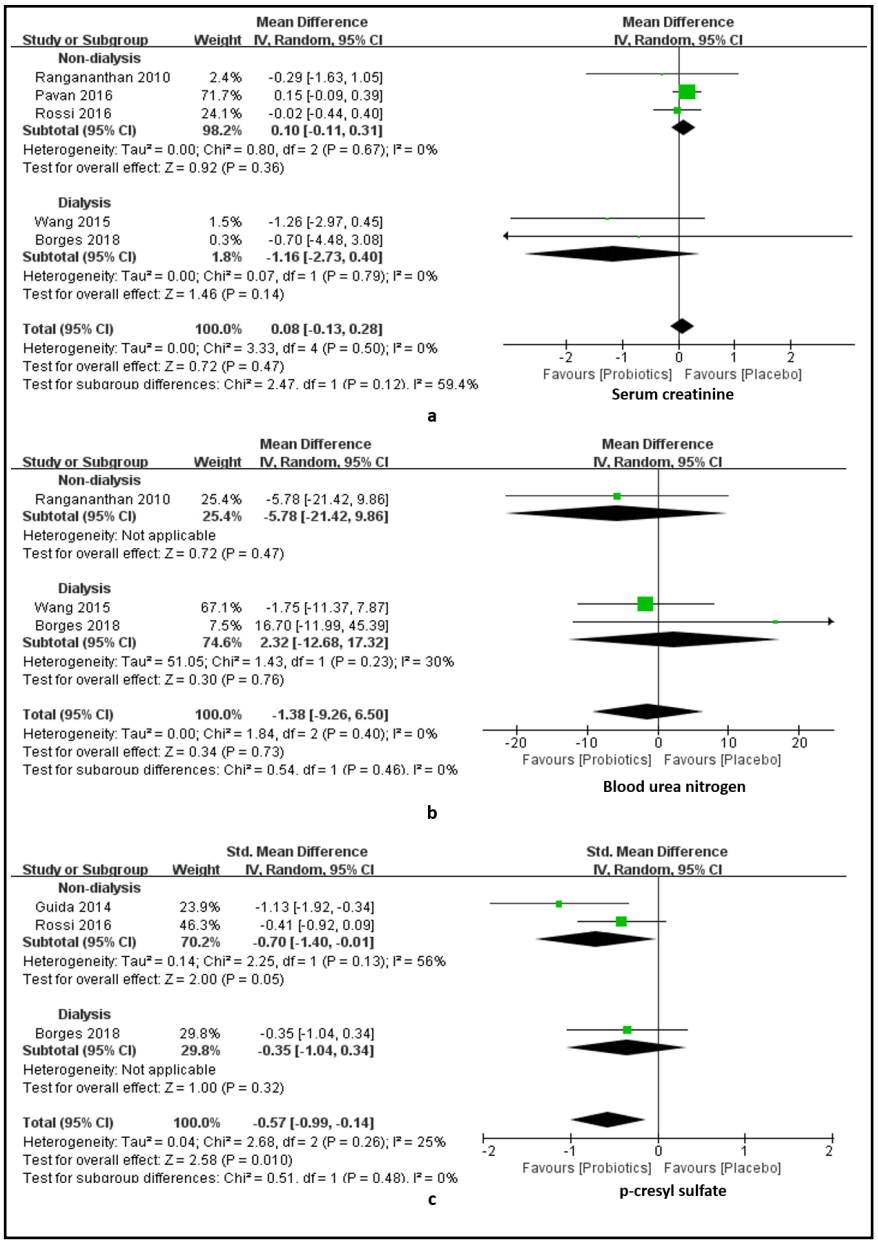

Fig. 3. Forest plots for comparisons of serum creatinine (Scr), blood urea (BUN) and p-cresyl sulfate (PCS) Five studies reported data of Scr (a) and three studies reported data of BUN (b) in experimental and control chronic kidney disease (CKD) patients. Neither Scr (P = 0.47, 95% CI, -0.13 to 0.28, I^2 = 0%) nor BUN (P = 0.73, 95% CI, -9.26 to 6.50, I^2 = 0%) changed significantly after the probiotics supplementation compared with the placebo treatment in main analysis and subgroup analysis. PCS of chronic kidney disease patients showed a significant decrease after treated with probiotics (P = 0.01, SMD -0.57, 95% CI, -0.99 to -0.14, I^2 = 25%, c) in main analysis and subgroup analysis.

Levels of serum IL-6 in patients with CKD were increased after taking probiotics

Data of 46 probiotics-treated patients and 47 placebo-treated patients were reported in three studies [11, 17, 20]. No significant difference in Hb was found between the two groups of patients with CKD (P = 0.55, 95% CI, -0.48 to 0.91, I^2 = 20%, Fig. 4a). In subgroup analysis, no trend of decreasing of Hb was found in patients with dialysis (P = 0.18, 95% CI, -0.24 to 1.29, I^2 = 0%, Fig. 4a).

As for IL-6, data of 68 patients in the probiotics group and 66 patients in the control group of three studies [10, 17, 22] were reviewed. A significant increase of serum IL-6 were found in the probiotics group (P = 0.03, 95% CI, SMD 0.37, 0.03 to 0.72, I^2 = 0%, Fig. 4b), as well as in patients with dialysis (P = 0.04, 95% CI, 0.03 to 0.97, I^2 = 0%, Fig. 4b).

Only three studies [11, 17, 19] reported the outcomes of CRP, and data of 53 patients in the probiotics group and 53 patients in the control group were compared (P = 0.78, 95% CI, -3.81 to 5.09, I^2 = 78%, Fig. 5a). Because the I^2 = 78% is higher than 50% [15], which

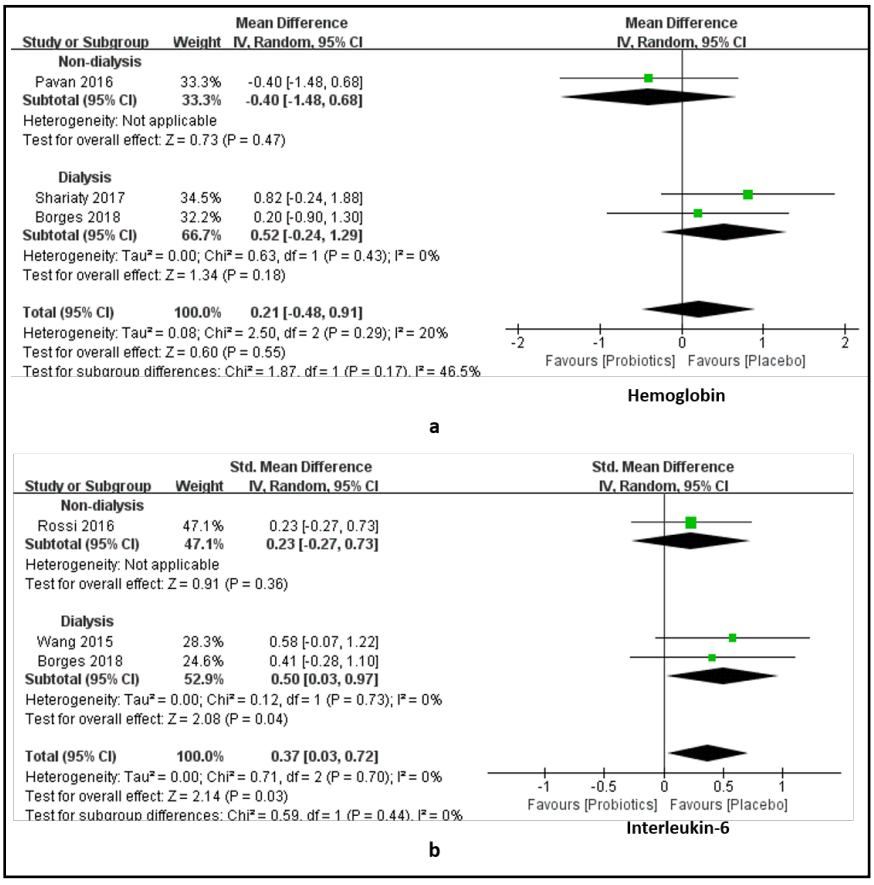

Fig. 4. Forest plots for comparisons of hemoglobin (Hb) and interleukin (IL)-6. Meta-analysis of Hb showed no statistical difference between probiotics and placebos groups (P = 0.55, 95% CI, -0.48 to 0.91, I^2 = 20%) (a). IL-6 of probiotics groups showed an increase (P = 0.03, SMD 0.37, 95% CI, 0.03 to 0.72, I^2 = 0%) (b). The same results were shown in main analysis and subgroup analysis.

represents an obvious heterogeneity, a sensitivity test was done. Even after Rangananthan and Shariaty's studies were excluded respectively, obvious heterogeneities were still evident (I^2 = 80% and I^2 = 78% respectively, Figs. 5b and 5c). However, after excluding Borges' study, the I^2 value fell to 28% (Fig. 5d), which means that the CRP data of Borges was the origin of heterogeneity. Hence, in sensitivity test, no difference was found in CRP between the probiotics and control groups (P = 0.49, 95% CI, -6.45 to 3.11, I^2 = 28%, Fig. 5d).

Discussion

In this meta-analysis, we assessed the therapeutic effectiveness of probiotics on CKD patients. Eight RCTs including 261 subjects were identified. Our primary finding is that probiotics could reduce the level of PCS, which is a major kind of urea toxins caused by dysbacteriosis. While the level of IL-6, a pro-inflammatory factor, was increased in patients with CKD in the intervention group. We found no significant changes of Scr, BUN and Hb after probiotics supplementation.

In CKD patients, non-*p*-cresol producing bacteria decreases [23]. The accumulation of PCS acts on organic anion transporter (OAT) on tubular epithelial cells to upregulate protein kinase C (PKC) and phosphoinositide 3-kinase (PI3K) pathways, which in turn activate reactive oxygen species (ROS) [24]. Then ROS subsequently stimulates the expression of inflammatory cytokines resulting in the nephrotoxicity and tubular fibrosis [25]. Some researchers considered probiotics as a promising adjuvant therapy for patients with CKD by reducing urea toxins especially PCS [8]. The results of our review is partially consistent with Rossi's [26] and Thongprayoon's meta-analyses [27]. Rossi and colleagues provided a supportive evidence that probiotics could reduce PCS of CKD patients. In Thongprayoon's

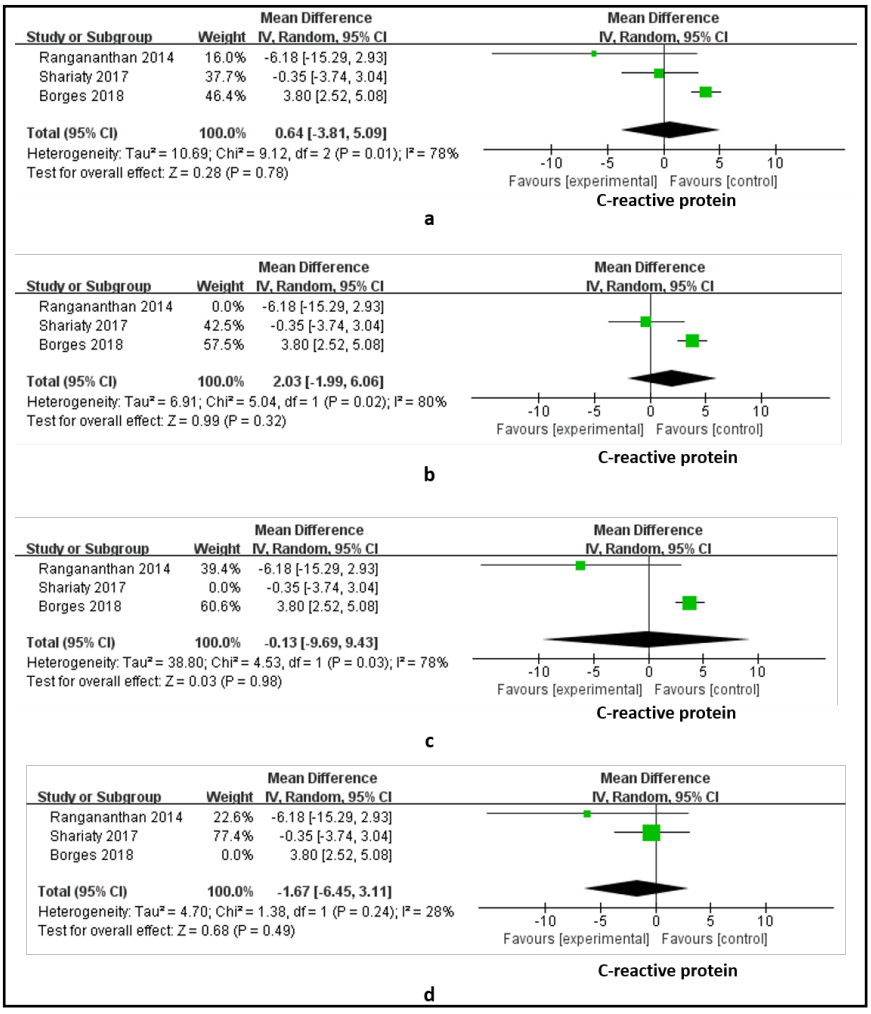

Fig. 5. Forest plots for comparisons of C-reactive protein (CRP). Three studies reported the results of CRP. However, the heterogeneity was obvious in the meta-analysis (I^2 = 78%) (a). Thus, a sensitivity analysis was done. After excluding Ranganathan's (b) and Shariaty's (c) studies, respectively, the I^2 was still higher than 50%. When Borges's study was excluded, I^2 was 28%, and no significant difference was shown in the probiotics group compared with the controls (P = 0.49, 95% CI, -6.45 to 3.11, I^2 = 28%) (d).

meta-analysis, a decreasing trend of PCS was found in CKD patients without dialysis after taking probiotics [27]. Other non-RCTs also reported that probiotics could change urinary *p*-cresol excretion and fecal *p*-cresol composition to some extent [28, 29]. A 3-year longitudinal study indicated that a 5μmol/L increase of PCS might be associated with a 17% increased risk of rapid progression to dialysis in pre-dialysis CKD patients [30]. Another study with a larger sample size also supported the positive relationship of PCS and independent cardiovascular events [31]. Thus, more longitudinal clinical trials with even larger sample size are needed to investigate the positive effects of probiotics on decreasing progression and complication risks of CKD.

IL-6 could act as both pro- and anti-inflammatory cytokines. Burton and his colleagues found a decrease of IL-6 in healthy young men after taking 2-week probiotics yogurt [32]. In inflammatory bowel diseases, probiotics were also verified to reduce IL-6 and inflammatory status [33]. However, it has been suggested that probiotics may increase the IL-6 level and further protect the intestinal epithelial barrier [34], which is consistent with our results. In fact, the balance between the role of IL-6 as pro- or anti-inflammatory cytokine is associated with signal transducers and activators of transcription (STAT) 1 and STAT3. IL-6 could activate STAT1, which is described to activate nuclear factor κB (NF-κB) [35], as well as STAT3, which is described to suppress the activation of NF-κB [36]. Thus, the role of IL-6 in probiotics supplementation may be affected by various factors, such as strains of probiotics, and further studies are warranted.

Except for PCS, probiotics had no significant effect on Scr. Due to the lack of data, estimated glomerular filtration rate was not included as an outcome. Moreover, stratified analyses according to Scr levels and dialysis status were not performed in any of the selected studies. Further studies are warranted to investigate the effect of probiotics in CKD patients with different Scr levels. As for BUN, results of three included trials were inconsistent. Borges found an increasing trend of BUN in patients treated with probiotics, which authors interpreted it as the influence of food intake [17]. While the other two studies found that the level of BUN was either decreased or unchanged after probiotics [10, 21]. Neither of the three RCTs stated the step of food intake recording, so other factors may result in the great differences among conclusions on BUN, such as the CKD stage and dialysis status. Both Wang's and Rangananthan's studies excluded subjects with antibiotic drug history, whereas Borges did not. All the differences would lead to discrepancies of results. Since the number of trials are limited, subgroup analysis was not conducted in our meta-analysis. Thus, further studies are necessary to investigate the BUN changes after the probiotics supplementation.

As anemia is an important complication of CKD, levels of Hb were analyzed as an outcome in our study. Shariaty's team reported that Hb of both probiotics and placebo groups were increasing during the experiment, and compared with placebo group, the increase rate of intervention group is higher [11]. Nevertheless, no significant changes of Hb levels were found after taking probiotics, which is consistent with the other two studies [17, 20]. A clinical trial on healthy adults also showed a slight change of Hb in the probiotics supplementation [37].

Another important biomarker of inflammation analyzed in our study is CRP. CRP has been found to increase in CKD patients than healthy people [11]. Many researchers have assumed that the probiotics therapy could decrease the inflammatory status of CKD patients. Our meta-analysis shows insignificant difference after taking the probiotics, which is inconsistent with Thongprayoon's meta-analysis [38]. They demonstrated a significant reduction in CRP level in the of CKD patients with dialysis [38]. However, some non-RCTs were included in Thongprayoon's report, such as Simenhof's [39] and Nakabayashi's [40] studies, which might influence the quality of results. In the analysis of CRP, significant heterogeneity was found to be caused by Borges' data (Figs. 5a-5c). Because the baseline data of CRP were incomparable between the experimental and the control groups, the results were not reliable even if the data at the terminal point of the study were considered as the same [17]. In this regard, it is important to examine whether baseline data of studied subjects are comparable in RCTs.

Half of the studies stated that adverse events of probiotics were recorded [10, 19, 21, 22]. Three of them reported no adverse events during the study, and one study reported a case of vomiting and nausea during the probiotics supplementation [19]. One patient developed myocardial infarction during sleep. The authors considered the severe adverse event was caused by heavy smoking as well as bad compliance to follows-up [19]. Taken together, the probiotics supplementation is largely relatively safe.

The antibiotic medication history is also an important impact factor in the probiotics supplementation and may have an impact on the study results, especially urea toxins. However, few studies reported how antibiotics may influence study results during the RCTs. In our meta-analysis, researchers of three studies defined antibiotic medication history as one of excluding criteria during the subject recruitment [10, 11, 21]. Rossi's team compared the statistical results between all completers and antibiotic-free completers as a prespecified sensitivity analysis [22]. They found that probiotic treatment resulted in a potentially clinically important 22%-28% reductions of PCS in analysis of antibiotic-free patients [22]. Hence more attention should be paid to the effect of antibiotics in the probiotics studies.

Our meta-analysis was focused on the effects of probiotics on CKD. A most recent meta-analysis was conducted by Thongprayoon et al, who reviewed the effects of probiotics on inflammation and urea toxins in CKD patients with dialysis only [38]. Apart from those parameters, we employed Hb and IL-6 to evaluate whether probiotics could improve renal

anemia and proinflammatory status. To ensure the quality of our meta-analysis, we included RCTs only. Despite these advantages, our study has some limitations. First, the number of RCTs included in our study is limited, since probiotics research is an emerging research field in CKD. The limited number of studies made subgroup analysis of CKD5 with or without dialysis difficult, even impossible. Especially in the meta-analysis of Hb and Scr, although no heterogeneity was found in total (Fig. 3a and Fig. 4a), tests for subgroup differences displayed marked heterogeneity (I^2 = 59.4% for Scr and I^2 = 46.5% for Hb), suggesting differences between CKD patients with and without dialysis. Since the number of studies was less than 10, meta-regression analysis to explore the origin of heterogeneity was not possible [15]. Thus, update of this meta-analysis remains necessary for further discussing the effect of probiotics on CKD5 with or without dialysis separately. Second, the sample size of studied subjects was small, which may increase the reporting bias and reduce the quality of meta-analysis. Finally, the treatment duration was relatively short and no longitudinal study was included, which may lead to reporting bias as well.

Conclusion

Although no significant changes of Scr, BUN and Hb were found after treatment, our meta-analysis provides evidence that the probiotics supplementation is likely to reduce PCS and increase IL-6 of patients with CKD. Results of our study suggest that probiotics may be a promising adjuvant therapy for CKD.

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Data available: All the original data of this study could be downloaded at https://osf.io/azh92/.

Disclosure Statement

The authors declare no conflict of interests.

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