TITLE

Effect of Synbiotic Therapy on Renal Function Outcomes in Patients with Chronic Kidney Disease Stage III–IV: A double-blind Randomized placebo-controlled Trial

NCT Number: Not yet

DATE: 15/09/2026

To

The Principal

Chittagong Medical College

Chattogram.

Subject: Application for the approval of Thesis Protocol with the title, **"Effect of Synbiotic Therapy on Renal Function Outcomes in Patients with Chronic Kidney Disease Stage III–IV: A Randomized Placebo-Controlled Trial"**

Sir,

With due respect and humble submission, I would like to state that I am a student of MD (Nephrology) Phase B, Resident, at BMU, Dhaka. As per requirement of the course, I would like to perform my research work on the thesis with the above-mentioned title under the direct supervision of Prof. Dr. Md Nurul Huda, Professor and Head, Department of Nephrology, Chittagong Medical College and Hospital, Chattogram, Bangladesh.

I therefore, like to request you to approve my protocol so that I can commence my work in your esteemed institute to complete my thesis in due time.

Obediently Yours

Dr. Md. Rezwanul Haque

MD (Nephrology) Phase B, Resident

Department of Nephrology

Chittagong Medical College and Hospital

Chattogram, Bangladesh

CMC Ethical Review Committee
Chittagong Medical College
Chattogram-4000, Bangladesh
Tel-031619400, Fax-630180

Application for Ethical Clearance of Studies for post-Graduate Thesis

1.	Name of the applicant	:	**Dr. Md. Rezwanul Haque**
2.	Course	:	MD (Nephrology) Phase B, Resident
3.	Category	:	Government
4.	Title of the study	:	Effect of Synbiotic Therapy on Renal Function Outcomes in Patients with Chronic Kidney Disease Stage III–IV: A Randomized Placebo-Controlled Trial in Bangladesh
5.	Type of the study	:	Double-blind, randomized, placebo-controlled trial
6.	Duration of the study	:	One and half year from acceptance of protocol.
7.	Any collaboration	:	No
8.	Conflict of interest	:	None
9.	Name of the Guide	:	Prof. Dr. Md Nurul Huda, Professor and Head, Department of Nephrology Chittagong Medical College and Hospital
10.	Name of the Co-Guide	:	Dr. Rosanna Bintey Kamal MBBS, MD (Nephrology) Assistant professor Department of Nephrology Chittagong Medical College and Hospital.

Check Documents being submitted herewith to committee:

1.	Summary	**:** Attached
2.	Umbrella proposal initially	**:** NA
3.	Protocol and CRF	**:** Attached
4.	Informed consent form for subject	**:** Attached
5.	Verbal consent form for subjects	**:** NA
6.	Procedure for Maintaining Confidentiality	**:** Attached
7.	Schedule of the study	**:** Attached

Declaration

I agree to obtain approval of the Ethical Review Committee for any changes involving the rights and welfare of subjects or any changes of the methodology before making any such changes.

……
Principal investigator/Student

Dr. Md. Rezwanul Haque
MD (Nephrology) Phase B, Resident
Department of Nephrology
Chittagong Medical College and Hospital
Chattogram, Bangladesh

Forwarding from the Guide

……
Guide

Prof. Dr. Md Nurul Huda
Professor and Head
Department of Nephrology
Chittagong Medical College and Hospital
Chattogram, Bangladesh

CHITTAGONG MEDICAL COLLEGE

Application for the Ethical review of thesis protocol

1.	Name of the applicant	:	Dr. Md. Rezwanul Haque
2.	Course	:	MD (Nephrology) Phase B, Resident
3.	Category	:	Government
4.	Title of the study	:	Effect of Synbiotic Therapy on Renal Function Outcomes in Patients with Chronic Kidney Disease Stage III–IV: A Randomized Placebo-Controlled Trial.
5.	Type of the study	:	Double-blind, randomized, placebo-controlled trial
6.	Duration of the study	:	One and half year
7.	Any collaboration	:	No
8.	Conflict of interest	:	None
9.	Name of the Guide	:	Prof. Dr. Md Nurul Huda, Professor and Head, Department of Nephrology Chittagong Medical College and Hospital
10.	Name of the Co-Guide	:	Dr. Rosanna Bintey Kamal MBBS, MD (Nephrology) Assistant professor Department of Nephrology Chittagong Medical College and Hospital.
11.	Signature of the Guide	:	
12.	Signature of the Co-Guide	:	
13.	Submission date	:	
14.	Signature of the Student	:	

For Official use: Serial No: Comment:	Received on:	Reviewed on:

Member-Secretary ERC

Chairperson ERC

Circle the appropriate answer to each of the following

(If not Applicable write NA)

1. Source Population:			**4. Are subjects clearly informed about:**		
(a) Ill Subjects	**√**Yes	No	(a) Nature and purpose of the study	**√**Yes	No
(b) Non* ill subjects	Yes	**√**No	(b) Procedures to be followed including alternatives used	**√**Yes	No
(c) Minors or persons under guardianship	Yes	**√**No	(c) Physical risks	**√**Yes	No
			(d) Private questions	**√**Yes	No
2. Does the study involve:			(e) Invasion of the Body	**√**Yes	No
(a) Physical risks to subjects	Yes	**√**No	(f) Benefits to be Derived	**√**Yes	No
(b) Social risks	Yes	**√**No	(g) Right to refuse to participate or withdraw from the study	**√**Yes	No
(c) Psychological risks to subjects	Yes	**√**No	(h) Confidential handling of data	**√**Yes	No
(d) Discomfort to Subjects	Yes	**√**No	(i) Compensation where there are risks or loss of working time or privacy is involved in any particular procedure	**√**Yes	No
(e) Invasion of the body	**√**Yes	No			
(f) Invasion of privacy	Yes	**√**No			
(g) Disclosure of information damaging to subject or others	Yes	**√**No			
			5. Will informed consent be required		
			(a) From subject	**√**Yes	No
			(b) From parent or Guardians	Yes	**√**No
3. Does the study involve:					
(a) Use of records (hospital, medical, death, birth or other)	Yes	**√**No	**6. Will precautions will be taken to protect anonymity of subjects**	**√**Yes	No
(b) Use of fetal tissue or abortus	Yes	**√**No			
(c) Use of organs or body fluids	Yes	**√**No			

Chittagong Medical College
Chattogram-4000, Bangladesh
Tel-031619400, Fax-630180
Research Proposal
For Post Graduate Thesis/Dissertation

Part - A

1.	Title of the study	:	Effect of Synbiotic Therapy on Renal Function Outcomes in Patients with Chronic Kidney Disease Stage III–IV: A Randomized Placebo-Controlled Trial.
2.	Name of the applicant	:	Dr. Md. Rezwanul Haque
3.	Course	:	MD (Nephrology) Phase B, Resident
4.	Place of Study	:	Department of Nephrology, Chittagong Medical College Hospital, Chattogram.
5.	Sponsoring	:	Not Applicable
6.	Duration of the study	:	One and half year from acceptance of protocol
7.	Date of Commencement	:	
8.	Date of completion	:	
9.	Total cost	:	616000/=
10.	Other support of proposed research		
	i. In this research project being supported by any other source?		No
	ii. Has an application for funding of this project has been submitted to any other organization (s)?		No
11.	Date of submission	:	
12.	Signature of student	:	
13.	Signature of Guide	:	
14.	Endorsement of the course coordinator		
	Name and Signature:		
	Designation:		
	Official Seal:		

Part – B

Student's Information Sheet

1. Name : Dr. Md. Rezwanul Haque
   Designation : MD (Nephrology) Phase B, Resident
   Official address with telephone & mail : Department of Nephrology, Chittagong Medical College Hospital, Chattogram
   Phone: 01710457014
   Email:drrezwanulhaque@gmail.com
   Present residential address : 54B/2, Road#6, Nasirabad Housing Society
2. Academic Background :

Degree	**Institute**	**University/Board**	**Field**	**Year**
M.B.B.S	**Shaheed Ziaur Rahman Medical College**	**Rajshahi**	Passed	**2014**

3. Field of study : Nephrology
4. Research experience : None
5. Percentage of time to be devoted to this project : 50%
6. Number of Scientific Publication : None

…….

Signature of the Student

PART-C
Summary

Background: Chronic kidney disease (CKD) represents a growing global public health challenge, with rising prevalence in low and middle-income countries, including Bangladesh. Progressive renal dysfunction is frequently accompanied by gut microbiota alterations, leading to increased intestinal permeability and systemic inflammation through the gut–kidney axis. Accumulation of uremic toxins derived from dysbiotic microbiota has been implicated in CKD progression. Synbiotic therapy, combining probiotics and prebiotics, has been proposed as a strategy to restore microbial balance and reduce inflammatory burden.

Objective: To evaluate effect of Synbiotic Therapy on Renal Function Outcomes in Patients with Chronic Kidney Disease Stage III–IV.

Materials and Methods: This Double-blind, randomized placebo-controlled trial will be conducted in the Department of Nephrology, Chittagong Medical College Hospital, Bangladesh. Approximately 96 adult non-dialysis CKD stage III–IV patients will be enrolled. Participants will be randomly allocated into two arms of treatment. The experimental arm and the control arm. Both arm with receive standard care according to KDIGO recommendations. The experimental arm will receive synbiotic therapy and the control arm with receive placebo in addition to standard therapy. Clinical and laboratory assessments will be performed at baseline, after 1.5 months and after 3 months. Outcome measures are change in S. Creatinine, e-GFR, Blood Urea Nitrogen (BUN), C-Reactive protein (CRP), and Gastrointestinal symptom dynamics using Gastrointestinal System Rating Scale (GSRS).

Data Analysis Plan: An intention-to-treat or per protocol approach will be applied as applicable. Parametric or non-parametric tests will be applied as appropriate. Repeated-measures analysis will assess longitudinal changes.

Conclusion: The expected findings may clarify the potential role of gut microbiota modulation as an adjunct in CKD management in Bangladesh and similar settings and may inform future multicenter trials.

Keywords: Chronic Kidney Disease; Synbiotic; Gut–Kidney Axis

PART-D

1. Introduction:

Globally, chronic kidney disease (CKD) has emerged as a major non-communicable disease. Recent estimates suggest that approximately 9–13% of the world's population is affected, translating into hundreds of millions of individuals living with impaired renal function (Guo et al., 2025). CKD now ranks among the leading causes of years of life lost worldwide, with a disproportionate impact in low and middle-income countries (LMICs) (Ying et al., 2024).

Stages III and IV CKD represent a critical window in the disease trajectory. During this period, progressive nephron loss, glomerulosclerosis, tubulointerstitial fibrosis, and microvascular rarefaction contribute to a steady decline in renal function (Reiss et al., 2024). Although renal replacement therapy remains the definitive treatment for ESRD, it is costly, resource-intensive, and associated with significant co-morbidities. Therefore, interventions aimed at slowing progression during earlier stages are central to nephrology practice.

Current standard therapy for CKD focuses primarily on control of blood pressure, often with renin–angiotensin–aldosterone system (RAAS) blockade, glycaemic control in diabetic nephropathy, lipid management, dietary protein restriction as indicated, correction of anemia, and management of mineral and bone disorders (KDIGO, 2013; Navaneethan et al., 2023). While these strategies reduce cardiovascular risk and modestly slow renal function decline, many patients continue to experience progressive deterioration despite optimized therapy. This residual risk suggests the involvement of additional pathophysiological mechanisms not fully addressed by conventional approaches.

Increasing attention has been directed towards systemic inflammation and the accumulation of gut-derived uremic toxins as contributory drivers of CKD progression. Elevated circulating levels of indoxyl sulfate, p-cresyl sulfate, and trimethylamine-N-oxide have been associated with oxidative stress, endothelial dysfunction, and further

nephron injury (Tsuji et al., 2024; Vaziri et al., 2013). Chronic low-grade inflammation, reflected by elevated C-reactive protein (CRP) and pro-inflammatory cytokines, is consistently observed in moderate to advanced CKD and is independently associated with adverse renal and cardiovascular outcomes (Carrero et al., 2010). These findings indicate that CKD is not solely a hemodynamic or metabolic disorder, but also a systemic inflammatory condition with complex inter-organ interactions. Within this broader pathophysiological framework, disturbances in gut microbiota composition, often referred to as dysbiosis have been increasingly implicated. The recognition of a bidirectional "gut–kidney axis" has introduced new perspectives on CKD progression and potential therapeutic targets.

Healthcare systems in Bangladesh operate under resource limitations, especially in the context of renal replacement therapy. Hemodialysis services are concentrated in urban tertiary centers, and out-of-pocket expenditure remains substantial (Mostafi et al., 2023). Consequently, many patients present at advanced stages, and long-term dialysis may not be financially sustainable for a significant proportion of the population. In such settings, strategies that may delay progression from stage III–IV CKD to ESRD are of considerable clinical and public health relevance.

Nutritional patterns, recurrent infections, and environmental factors prevalent in South Asian contexts may further influence CKD progression and inflammatory burden (Vijayan et al., 2023). Diets relatively high in refined carbohydrates and low in dietary fiber may alter gut microbial diversity. Additionally, frequent exposure to antibiotics can disrupt microbial equilibrium, potentially exacerbating dysbiosis. These contextual elements intersect with metabolic comorbidities to shape disease trajectories.

The concept of the gut–kidney axis posits that the gastrointestinal tract and kidneys engage in bidirectional communication through metabolic, immunologic, and endocrine pathways. Uremia has been shown to disrupt tight junction proteins in the intestinal epithelium, increasing permeability and facilitating translocation of endotoxins into systemic circulation (Vaziri et al., 2012). This process contributes to chronic inflammation, oxidative stress, and vascular dysfunction. Conversely, modulation of gut microbiota may influence the production of toxin precursors and inflammatory mediators. Emerging microbiome-directed therapies, including

probiotics, prebiotics, and their combination as synbiotics have been proposed as potential strategies to restore microbial balance and reduce toxin generation.

Accumulating evidence suggests that CKD is associated with significant alterations in gut microbial composition. Experimental and clinical studies have reported reduced populations of Lactobacillus and Bifidobacterium species and increased abundance of urease- and uricase-producing bacteria in CKD patients (Vaziri et al., 2013). These microbial shifts favor proteolytic fermentation, leading to increased production of uraemic toxins.

Probiotics are defined as live microorganisms which, when administered in adequate amounts, confer a health benefit on the host (Gibson et al., 2017). Prebiotics are selectively fermented substrates that promote the growth or activity of beneficial microorganisms. Synbiotics combine both components, aiming to enhance microbial survival and colonization.

Mechanistically, probiotics may compete with toxin-producing bacteria, enhance short-chain fatty acid production, strengthen epithelial barrier function, and modulate immune responses. Prebiotics such as inulin and oligosaccharides serve as substrates for beneficial bacteria, promoting saccharolytic fermentation and reducing proteolytic toxin generation. Through these mechanisms, synbiotics may reduce circulating concentrations of uremic toxins and inflammatory mediators.

Several clinical investigations have evaluated microbiome-targeted interventions in CKD populations, though findings remain heterogeneous. Rossi et al. (2016) conducted a randomized controlled trial in non-dialysis CKD patients and observed reductions in serum p-cresyl sulfate following synbiotic supplementation, though changes in e-GFR were not statistically significant over short follow-up. Similarly, Guida et al. (2014) reported improvements in certain metabolic parameters and toxin levels in CKD patients receiving synbiotics. Recently, in another RCT Mitrović et al. (2023) confirmed that synbiotic treatment in CKD patients significantly modified gut microbiome, improved eGFR, and decreased level of high-sensitivity C-reactive protein.

A recent meta-analysis of 21 randomized controlled trials reported that probiotic or synbiotic supplementation significantly reduced BUN and CRP levels in CKD patients, although consistent improvement in serum creatinine was not demonstrated (Liu et al., 2024). Similarly, another meta-analysis showed reductions in inflammatory and oxidative stress markers and modest improvements in lipid profiles following microbial therapy (Zheng et al., 2020). However, individual trials have yielded heterogeneous findings; for example, the SYNERGY II randomized trial confirmed microbiota modulation but observed no clear renal functional benefit and even a small decline in eGFR over time (McFarlane et al., 2021).

Overall, current evidence suggests potential anti-inflammatory and metabolic benefits of synbiotic therapy in CKD, while its effect on renal function outcomes remains inconclusive and warrants further evaluation in well-designed trials involving non-dialysis stage III–IV populations.

Thus, the aim of this study to evaluate effect of symbiotic therapy on renal function outcomes in CKD stage III-IV patients.

2. Rationale:

The prevalence of CKD is increasing rapidly due to urbanization, lifestyle changes and delayed diagnosis. Conventional management strategies primarily aim to slow disease progression through blood pressure control, glycemic management, and renin-angiotensin system blockade; however, these approaches do not directly address the gut-kidney axis, which is now recognized as a significant contributor to CKD progression. Recent evidence suggests that gut dysbiosis in CKD leads to increased production of uraemic toxins, contributing to systemic inflammation, oxidative stress and further renal injury. In Bangladesh, where access to renal replacement therapy is limited and costly, identifying affordable adjunctive therapies is crucial. No adequately designed randomized, double-blind trial has evaluated synbiotic therapy in patients with CKD stages III-IV in Bangladesh. Population-specific factors such as dietary habits, environmental exposure and antibiotic use may influence gut microbiota and treatment response. Furthermore, there is a lack of locally relevant outcome data assessing renal function outcomes, uraemic toxin levels and inflammatory markers.

3.1 Research questions: Does adjunctive synbiotic therapy improve renal function outcomes in patients with chronic kidney disease stage III-IV compared with placebo?

3.2 Research Hypothesis: Adjunctive synbiotic therapy significantly improves renal function outcomes in patients with chronic kidney disease stage III-IV compared with placebo.

4. Objectives

General Objective

To evaluate the effect of adjunctive synbiotic therapy on renal function outcomes in patients with chronic kidney disease stage III–IV compared with placebo.

Specific Objectives

i) To assess and compare progression of CKD with changes of S. Creatinine, eGFR and BUN between intervention and placebo group.

ii) To compare the rate of e-GFR decline between intervention and placebo group.

iii) To evaluate and compare the effect of synbiotic therapy on systemic inflammation by C-Reactive protein(CRP) between two groups.

iv) To assess changes in Gastrointestinal symptoms using Gastrointestinal Symptom Rating Scale(GSRS) between two groups.

v) To evaluate the safety and adverse effects of synbiotic therapy.

5. Materials and method

5.1. Type of study: Double-blind, randomized, placebo-controlled trial.

5.2 Place of Study: Department of Nephrology, Chittagong Medical College Hospital, Chattogram, Bangladesh.

5.3 Study period: One and half year.

5.4 Study population: Chronic kidney disease stage III–IV patients.

5.5 Study groups: Eligible participants will be randomly assigned into two groups:

Experimental Group (Group-A):

Synbiotic therapy (1 capsule per day) along with Standard therapy according to KDIGO guideline will be administered for 3 months.

Control Group (Group-B):

Placebo(1 capsule per day) along with standard therapy according to KDIGO guideline will be administered for 3 months.

5.6 Sampling technique: All patients attending the nephrology department during the study period will be screened for eligibility. Screening will be performed using clinical records, laboratory reports, and direct patient interviews.

All eligible and consenting patients meeting the inclusion criteria during the recruitment period will be enrolled until the required sample size is achieved.

Randomization will be performed using a computer-generated random allocation sequence. Allocation concealment will be maintained through the use of sealed, opaque envelopes prepared prior to enrollment.

5.7. Inclusion criteria:

i. Age ≥18 years.
ii. Diagnosed chronic kidney disease stage III or IV as per KDIGO guidelines.
iii. No use of probiotics, prebiotics, or synbiotics within the previous two weeks.
iv. Willingness to provide written informed consent.

5.8 Exclusion criteria

i. Current use of immunosuppressive therapy.
ii. Presence of chronic diarrhoeal disease, inflammatory bowel disease, or history of major bowel surgery.
iii. Fever or any acute illness within the study period.
iv. Pregnancy or lactation.
v. Known hypersensitivity to probiotic or synbiotic formulations.
vi. Acute kidney injury or requirement for dialysis at the time of screening.

5.9. Sample size: The sample size will be calculated based on the primary outcome measure, namely change in eGFR between the intervention and control groups after three months of follow-up. The formula for comparison of two independent means will be used:

$$n = \frac{2(Z_{\alpha/2} + Z_{\beta})^2 \sigma^2}{d^2}$$

Where:

- n= required sample size per group
- $Z_{\alpha/2}$= standard normal deviate at 5% level of significance (1.96)
- Z_{β}= standard normal deviate at 80% power (0.84)
- σ= pooled standard deviation of eGFR
- d= minimum clinically significant difference between groups

Based on previous randomized studies evaluating synbiotic therapy in CKD patients, a standard deviation of approximately 8 mL/min/1.73 m² for eGFR change has been

reported, and a difference of 5 mL/min/1.73 m² will be considered clinically meaningful (Mitrović et al., 2023).

Substituting the values:

$$n = \frac{2(1.96 + 0.84)^2(8)^2}{(5)^2}$$

$$n = \frac{2(2.8)^2(64)}{25}$$

$$n = \frac{2(7.84)(64)}{25}$$

$$n = \frac{1003.84}{25}$$

$$n \approx 40$$

Thus, approximately 40 participants will be required in each group. Considering a potential attrition rate of 20%, the final sample size will be adjusted to 48 participants per group. Therefore, a total of 96 participants will be recruited for the study.

5.10. List of variables

5.11. Sociodemographic Variables

1. Age (years)
2. Sex (male/female)
3. Residence (urban/rural)

1. Independent Variable (Exposure Variable)

Administration of synbiotic therapy (Yes/No)

Group allocation:

- Intervention group (Standard therapy + synbiotic capsule): Group-A
- Control group (Standard therapy + placebo): Group-B

2. Dependent Variables (Outcome Variables)

A. Primary Outcome Variable

Changes in serum creatinine, estimated Glomerular Filtration Rate (eGFR) and Blood Urea Nitrogen(BUN)

B. Scecondary Outcome Variables

i. Changes in C-Reactive protein(CRP)

ii. Gastrointestinal symptoms improvement

-Measured using Gastrointestinal Symptom Rating Scale(GSRS)(Appendix A1)

iii. Adverse effects(increased flatulence, abdominal discomfort/pain, diarrhoea)

5.11 Operational definitions:

CKD: According to 2024 KDIGO guideline for CKD , CKD is defined as abnormalities of kidney structure or function, present for a minimum of 3 months, with implications for health . Criteria for chronic kidney disease (either of the following present for a minimum of 3 months)

1. Markers of kidney damage (1 or more):

a. Albuminuria (ACR ≥30 mg/g [≥3 mg/mmol])

b. Urine sediment abnormalities

c. Persistent hematuria

d. Electrolyte and other abnormalities due to tubular disorders

e. Abnormalities detected by histology

f. Structural abnormalities detected by imaging

g. History of kidney transplantation

- CKD Stage III:

  -CKD stage III will be defined as eGFR between 30–59 mL/min/1.73 m², calculated using the CKD-EPI equation.

- CKD Stage IV:

  -CKD stage IV will be defined as eGFR between 15–29 mL/min/1.73 m², calculated using the CKD-EPI equation.

Estimated Glomerular Filtration Rate (eGFR):

Estimated glomerular filtration rate will be calculated using the CKD-EPI equation based on serum creatinine, age, and sex, and will be expressed in mL/min/1.73 m². It will be used as the primary indicator of renal function.

The CKD-EPI equation, expressed as a single equation, is:

GFR = 141 × min (Scr/κ,1)α × max(Scr/κ, 1)-1.209× 0.993Age × 1.018 [if female] × 1.159 [if black]

Scr is serum creatinine (mg/dL), κ is 0.7 for females and 0.9 for males, α is -0.329 for females and -0.411 for males, min indicates the minimum of Scr/κ or 1, and max indicates the maximum of Scr/κ or 1.

5. Synbiotic Therapy

Synbiotic therapy will be defined as the combined administration of probiotics and prebiotics in a single oral formulation. In this study, it will refer to a daily capsule containing Lactobacillus acidophilus (15 billion CFU), Bifidobacterium longum (15 billion CFU), Streptococcus thermophilus(15 billion CFU), along with xylo-oligosaccharide and inulin.

6. Standard Therapy

Standard therapy will be defined as guideline-directed medical management of CKD stage III–IV according to KDIGO recommendations, including blood pressure control , glycaemic control, dietary protein regulation, anemia management, and treatment of mineral and bone disorders.

15. Adverse Event

An AE is defined as any unwanted medical occurrence in a clinical trial patient during the study; the event does not necessarily have a causal relationship with that treatment. Therefore, an AE can be any unfavourable and unintended sign (including any clinically significant abnormal laboratory finding), symptoms, or disease, temporally associated with using a medicinal product. The common adverse events of symbiotic therapy is increased flatulence, abdominal discomfort/pain, diarrhoea.

Major adverse events will be defined as any event leading to hospital admission, withdrawal, or death.

Minor adverse events will be defined as minor if it does not require admission to the hospital or withdrawal of the drug.

Drug intolerance refers to the inability to tolerate the adverse effects of medication at a therapeutic or substantial dose. Participants experiencing major adverse event or drug intolerance will be withdrawn from the study.

5.12 Data collection instrument: A structured pretested case record form.

5.13 Study procedure:

Baseline Assessment

After eligibility will have been confirmed and written informed consent will have been obtained, baseline assessment will be performed. Sociodemographic data including age, sex, residence, will be recorded using a structured data collection form. Detailed clinical history including duration of CKD, presence of diabetes mellitus, hypertension, and medication history will be documented.

Physical examination will be conducted, and blood pressure, body weight, height, and body mass index (BMI) will be measured and recorded.

Baseline laboratory investigations will be carried out, including serum creatinine, BUN, CRP. eGFR based on serum creatinine will be calculated using the CKD-EPI equation.

Gastrointestinal symptoms assessment will be assessed by using Gastrointestinal Symptoms Rating Scale (GSRS), and scores will be calculated according to standardized scoring procedures. All baseline findings will be documented prior to allocation.

Allocation with Randomization

After completion of baseline assessment, participants will be randomly assigned to either experimental group or placebo group with a 1:1 allocation. Randomization will be carried out using online software (Research Randomizer Version 4.0 at https://www.randomizer.org).

Allocation concealment will be maintained through the use of sequentially numbered, sealed, opaque envelopes. Participants will then be assigned to either:

- The intervention group (standard therapy plus synbiotic), or
- The control group (standard therapy plus placebo)

Blinding: The study will be double blind, with both investigator and participants will be unaware of their treatment allocation.

Intervention

In the experimental group:

Standard treatment + 1 synbiotic capsule daily(45 billion CFU)

Duration: 3 months

In the control group:

Standard treatment + 1 Placebo capsule daily

Duration: 3 months

The synbiotic capsule will contain Lactobacillus acidophilus (15 billion CFU), Bifidobacterium longum (15 billion CFU), Streptococcus thermophilus (15 billion CFU), total 45 billion CFU along with xylo-oligosaccharide and inulin.

Participants will be instructed regarding the timing and method of capsule intake (Per oral with meal during breakfast). Adherence will be monitored through patient self-report and capsule count during follow-up visits.

Follow-Up

Participants will be followed for a total duration of three months.

After 1.5 months first follow up and after 3 months second follow up will be conducted.

In each follow up, Clinical reassessment and laboratory investigations including serum creatinine, e-GFR, BUN, and CRP will be repeated.

Gastrointestinal symptoms assessment will be assessed by using Gastrointestinal Symptoms Rating Scale (GSRS),

Any adverse events occurring during the study period will be documented and managed according to standard clinical practice. All collected data will be recorded systematically in the study database for subsequent statistical analysis.

5.14. Data analysis:

All collected data will be checked for completeness, consistency, and accuracy prior to analysis. Data will be entered into a secured database and subsequently analyzed using appropriate statistical software such as Statistical Package for the Social Sciences (SPSS) version 27.0 (IBM Corp., Armonk, NY, USA).

Descriptive statistics will be used to summarize baseline characteristics of the study participants. Continuous variables will be presented as mean ± standard deviation (SD) for normally distributed data or median with interquartile range (IQR) for non-normally distributed data. Categorical variables will be expressed as frequencies and percentages.

The normality of continuous variables will be assessed using statistical tests such as the Shapiro–Wilk test. Baseline comparability between the intervention and control groups will be evaluated using the independent samples Student's *t*-test for normally distributed continuous variables or the Mann–Whitney *U* test for skewed data.

Categorical variables will be compared using the Chi-square test or Fisher's exact test, as appropriate.

The primary outcome measure, change in e-GFR, will be analyzed by comparing mean changes between baseline and follow-up assessments across study groups. S.Creatinine will be measured in enzymatic method using Siemens Atellica Solution machine. e-GFR will be measured using CKD-EPI formula. Within-group comparisons over time will be performed using paired *t*-tests or Wilcoxon signed-rank tests depending on data distribution. Between-group differences in outcome changes will be evaluated using independent *t*-tests or corresponding non-parametric tests.

For repeated measurements obtained at baseline, at 1.5 months and at 3-months follow-up, repeated-measures analysis of variance (ANOVA) or a mixed-effects model will be applied to assess time, group, and interaction effects where assumptions are satisfied. When assumptions for parametric testing are not met, appropriate non-parametric alternatives will be used.

Secondary outcome variables, including BUN, CRP, and Gastrointestinal symptoms assessment assessed by using Gastrointestinal Symptoms Rating Scale (GSRS) will be analyzed using similar comparative statistical approaches. BUN will be measured in Urease, UV method, using Siemens Dimension EXL 200 Machine, CRP will be measured in Immunoturbidimetry method using Beckman Coulter AU480 machine.

An intention-to-treat (ITT) approach, whereby all randomized participants will be included in the analysis according to their allocated study groups irrespective of adherence or per protocol approach will be adopted. Missing data will be handled using appropriate statistical techniques such as last observation carried forward or multiple imputation, depending on the extent and pattern of missingness.

p value of less than 0.05 will be considered statistically significant. Effect sizes and 95% confidence intervals will be reported where applicable to facilitate interpretation of clinical relevance.

5.15. Expected outcomes: It is anticipated that the addition of synbiotic to standard therapy will be associated with improvement of S. Creatinine, eGFR, BUN compared to placebo over the study period. Improvements in secondary outcomes, including CRP, Gastrointestinal symptoms and no major adverse effect and intolerance are also expected.

5.16. The utilization of results: The findings of this randomized controlled trial will be utilized to inform clinical decision-making regarding adjunctive microbiota-targeted therapy in patients with CKD stage III–IV. If meaningful changes in renal function parameters, inflammatory markers, or gastrointestinal symptoms are observed, the results may contribute to the development of context-specific clinical recommendations for non-dialysis CKD management.

The study outcomes will be disseminated through submission to peer-reviewed national and international journals and presentation at scientific conferences in nephrology and internal medicine. The results will also be shared with the Department of Nephrology of the study institution to support evidence-informed practice.

All data generated from this study will be used strictly for academic and scientific purposes. Individual participant data will not be disclosed in any form that permits identification.

5.17. Ethical implication: The study will be conducted in accordance with the principles of the Declaration of Helsinki and relevant national ethical guidelines for biomedical research. Prior to initiation, ethical approval will be obtained from the Ethical Review Committee of Chittagong Medical College.

Written informed consent will be obtained from all participants before enrollment. The purpose, procedures, potential benefits, and possible risks of the study will be explained in clear and understandable language. Participants will be informed that participation will be voluntary and that they will have the right to withdraw from the study at any time without any effect on their standard medical care.

Confidentiality of participant information will be strictly maintained. Each participant will be assigned a unique identification code, and personal identifiers will not be

included in the data analysis or publications. Study documents will be stored securely, and access will be restricted to authorized research personnel only.

The synbiotic preparation to be used in the study is commercially available and generally regarded as safe. However, participants will be monitored for any adverse events throughout the study period. Any adverse reaction or clinical deterioration will be managed promptly according to standard medical practice. In cases where continuation in the study is deemed clinically inappropriate, the participant will be withdrawn and provided appropriate care.

No financial burden will be imposed on participants for study-related investigations directly related to the research protocol. No coercion or undue inducement will be applied during recruitment.

The study will ensure that participants in both groups will continue to receive standard guideline-directed therapy for CKD. Thus, no participant will be deprived of established treatment during the trial period.

All ethical safeguards will be implemented to ensure participant safety, scientific integrity, and responsible conduct of research throughout the study duration.

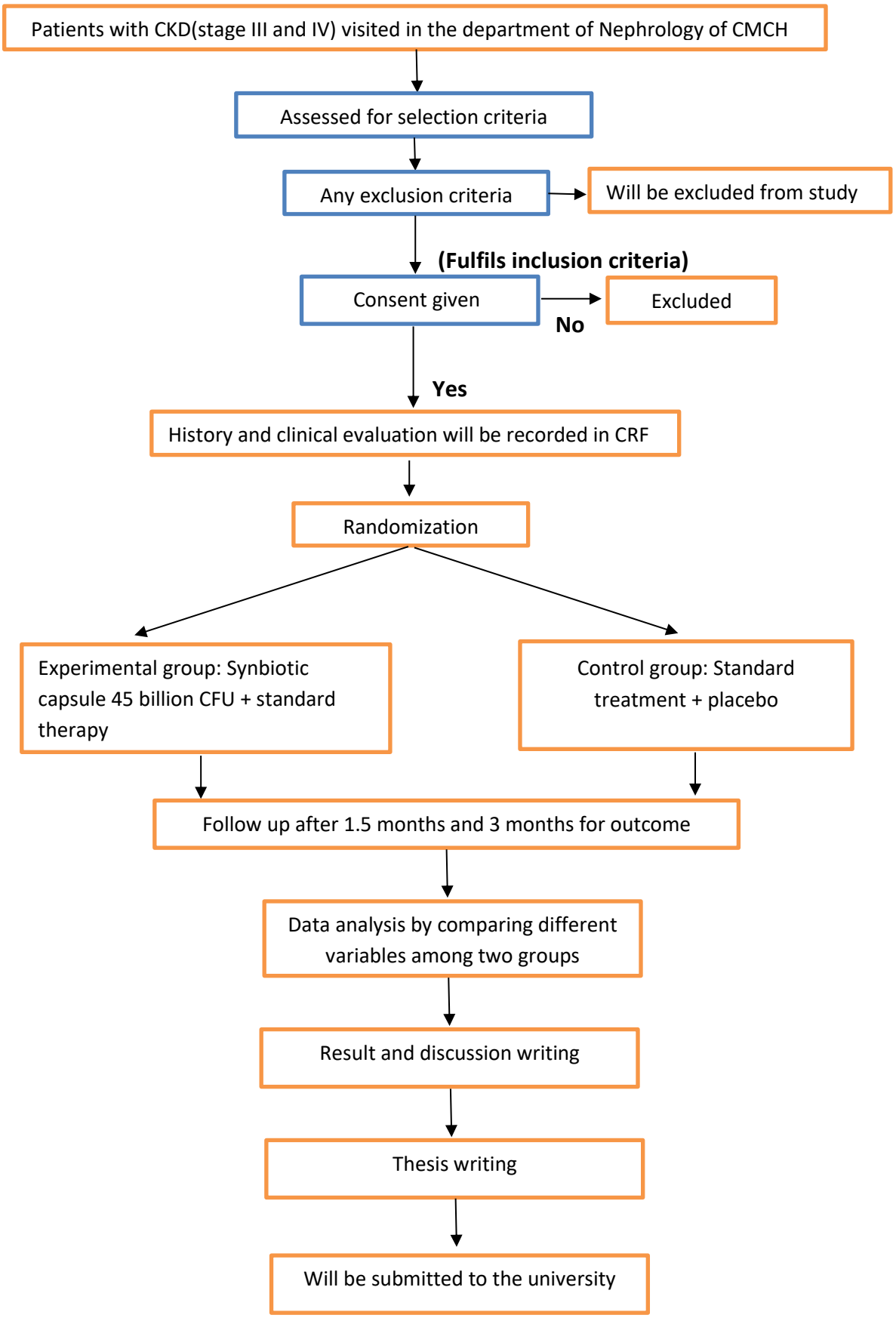
There will be no conflict of interest e,g no financial support from any organization.

5.18 Schedule(GANTT CHART)

TIME SCHEDULE

	1st	2nd	3rd	4th	5th -6th	7th-18th	19th-21th	20th-23th	24th
Problem definition									
Literature review									
Research design									
IRB clearance									
Data collection									
Data analysis									
Report writing									
Submission									

5.19 Study flow chart



5.20 References

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Part E

Budget:

Sl. No.	**Head of expense**	**Amount**
1	Research tools development	10,000/-
2	Office assistance for organizing the materials	5,000/-
3	Data collection	5000/-
4	Investigation cost	285000/-
5	Drug cost	240000/-
6	Honorarium allowance for assessor	20000/-
7	Data analysis	5000/-
8	Report composing	30,000/-
9	Printing	5,000/-
10	Stationaries	5,000/-
11	Transport/conveyance	1000/-
12	Miscellaneous	5000/-
	Total amount	**616000/-**

Appendix-A

Case record form

Protocol Title: "Effect of Synbiotic Therapy on Renal Function Outcomes in Patients with Chronic Kidney Disease Stage III–IV: A Randomized Placebo Controlled Trial"

Patient's identification			
1	Patient ID No.		
2	Contact no:		
3.	Study group: Group A: Experimental group Group B: Control group		
Demographic characteristics			
1	Age:	.....years	
2	Sex:	0=Female 1=Male	
3	Monthly income	.....BDT	
Clinical History			
1	Chronic Kidney Disease(CKD)	0=Stage III, 1= Stage IV	
2	Duration of CKD	..... years	
3	Co-morbidities	1= Diabetes Mellitus, 2= Hypertension 3= Cardiovascular disease, 4= Dyslipidaemia 5= Others	

Clinical Parameters		**At baseline**	**After 1.5 months**	**After 3 months**
1	Height(cm)			
2	Weight(kg)			
3	BMI(kg/m^2)			
4	Blood pressure(BP)			

Biochemical Parameters		At Baseline	After 1.5 months	After 3 months
1	Serum Creatinine (mg/dL)			
2	eGFR (mL/min/1.73m²)			
3	Blood Urea Nitrogen(BUN)(mg/dL)			
4	C-Reactive Protein(CRP)(mg/L)			
Gastrointestinal Symptom Rating Scale(GSRS)		**Score**		
1	At Baseline			
2	After 1.5 months			
3	After 3 months			
Adverse effects				
		At baseline	After 1.5 months	After 3 months
1	Flatulence/bloating			
2	Abdominal discomfort or pain			
3	Diarrhoea			
	0=Absent, 1=Present			

Signature of researcher **Signature of the Guide/Co-guide**

Gastrointestinal symptoms Rating Scale (GSRS): Appendix A1

5 main domains, each having Sub-domains:

1) Reflux Syndrome:

-Heartburn

-Acid regurgitation

2) Abdominal Pain Syndrome:

-Abdominal pain

-Hunger pains

-Nausea

3) Indigestion Syndrome:

-Borborygmus (stomach rumbling)

-Abdominal distension (bloating)

-Eructation (belching)

-Increased flatus (gas)

4) Diarrhea Syndrome:

-Loose stools

-Increased stool frequency

-Urgent need for defecation

5) Constipation Syndrome

-Hard stools

-Straining during defecation

-Feeling of incomplete evacuation

Each sub-domain has 7 rating points:

i) No discomfort ii) Minor discomfort iii) Mild discomfort iv) Moderate discomfort v) Moderately severe discomfort vi) Severe discomfort vii) Very severe discomfort

GSRS Mean Score interpretation:

Mean Score	Interpretation
1	No symptoms
2-3	Mild symptoms
4	Moderate symptoms
5-7	Severe symptoms

Appendix –B (i)

PARTICIPANT INFORMATION SHEET

Study Title:
Effect of Synbiotic Therapy on Renal Function Outcomes in Patients with Chronic Kidney Disease Stage III–IV: A Randomized Placebo-Controlled Trial

Principal Investigator:
Dr. Md. Rezwanul Haque, Department of Nephrology, Chittagong Medical College Hospital

1. Invitation to Participate

You are being invited to participate in a research study because you have been diagnosed with Chronic Kidney Disease (CKD) Stage III or IV. Before you decide whether to participate, it is important that you understand why the research is being conducted and what it will involve. Please read the following information carefully. You may ask any questions before making your decision.

Participation in this study is entirely voluntary.

2. Purpose of the Study

Chronic kidney disease may gradually worsen over time despite standard medical treatment. Recent research suggests that changes in intestinal bacteria may contribute to inflammation and kidney function decline. This study aims to evaluate whether adding a synbiotic (a combination of beneficial bacteria and prebiotics) to standard treatment may influence kidney function and inflammation levels.

3. Why You Have Been Selected

You have been selected because you:

1. Are aged 18 years or older
2. Have been diagnosed with CKD Stage III or IV
3. Are not currently on dialysis

Approximately 96 participants will be enrolled in this study.

4. Study Procedures

If you agree to participate:

- A baseline assessment will be performed, including medical history, physical examination, and blood tests.
- You will be randomly assigned (by chance, like a lottery) to one of two groups:

- One group will receive standard CKD treatment plus a daily synbiotic capsule for 3 months.
- The other group will receive standard CKD treatment and placebo.
- Follow-up visits will occur after 1.5 and 3 months.
- Blood tests will be repeated after 1.5 and 3 months.
- You will be asked to fill up a form to assess gastrointestinal symptom dynamics.
- The total study duration for each participant will be 3 months.

5. Possible Benefits

You may or may not directly benefit from participating in this study. The information gained from this research may help improve the understanding and management of CKD in the future.

6. Possible Risks or Discomforts

The synbiotic supplement used in this study is generally considered safe. Mild gastrointestinal symptoms such as bloating, abdominal discomfort, or diarrhea may occur in some individuals.

Blood sampling may cause mild discomfort, bruising, or rarely infection at the puncture site.

If any adverse event occurs, appropriate medical care will be provided.

7. Confidentiality

All information collected during this study will be kept strictly confidential. Your name will not appear in any report or publication. A unique study identification number will be assigned to you. Only the research team will have access to the data.

8. Voluntary Participation and Right to Withdraw

Your participation is voluntary. You may refuse to participate or withdraw at any time without affecting your regular medical care. Your decision will not influence your treatment at this hospital.

9. Cost and Compensation

You will not be required to pay for study-related investigations directly related to the research protocol. No financial incentive will be provided for participation.

10. Contact Information

If you have any questions about the study, you may contact:

Principal Investigator: Md. Rezwanul Haque

Phone: 01710457014

For questions regarding your rights as a research participant, you may contact the Ethical Review Committee (ERC) of Chittagong Medical College.

Consent form

"Effect of Synbiotic Therapy on Renal Function Outcomes in Patients with Chronic Kidney Disease Stage III–IV: A Randomized Placebo-Controlled Trial.
"

After being fully informed about the objectives, consequences of the study and any right to withdraw me from the study at any time for any purpose whatsoever, I am ---------- at this moment giving consent to participate in the study conducted by Dr. Md. Rezwanul Haque, MD (Nephrology) Phase-B student, Chittagong Medical College.

I fully recognize that my participation in this study will generate valuable medical information that might be used for the interest of patients in future.

In this research, if any adverse effects of drugs are found, investigators will take immediate measures for treatment.

I shall try my best to comply with the instruction given by the investigator throughout the whole period of study.

Signature/ Thumb impression
of the subject
Date.....

Signature/Thumb impression….
of the patient's guardian
Date……

Signature /Thumb impression
of the witness
Date.....

Signature /Thumb impression
of the Investigator
Date……

Data and Safety Monitoring Board

Title: "**Effect of Synbiotic Therapy on Renal Function Outcomes in Patients with Chronic Kidney Disease Stage III–IV: A Double blind Randomized Placebo-Controlled Trial"**

Name of the researcher: This study is being conducted by Dr. Md. Rezwanul Haque, Phase-B Resident, MD (Nephrology), Department of Nephrology, Chittagong Medical College.

Place of the study: Department of Nephrology, Chittagong Medical College Hospital, Chattogram.

Type of Study: Randomized Placebo-Controlled Trial

Members of the board:

• ……. Professor, Department of Nephrology Chittagong Medical College Hospital, Chattogram, Bangladesh

• ……. Associate Professor, Department of Nephrology Chittagong Medical College Hospital, Chattogram, Bangladesh

• ……. Assistant Professor, Department of Nephrology Chittagong Medical College Hospital, Chattogram, Bangladesh

DATA AND SAFETY MONITORING PLAN

Once a protocol is referred to the data and safety monitoring committee (DSMC). The first steps for the principal investigator to develop a Data and Safety Monitoring Plan (DSMP). A DSMP is a written plan that specifies a system for appropriate study oversight to ensure: (1) safety of clinical research subjects, (2) validity and integrity of research data, and (3) appropriate termination study. Once the plan elements listed here are submitted to the DSMC, the DSMC will work with the investigator to finalize the plan and assure that it is implemented.

Enrollment of the study participants by months:

Month	**Approached**	**Eligible**	**Randomized**	**Withdrawn**	**Actual #**	**Cumulative #**
1						
2						
3						
4						

Subject Status:

Patient ID	**Date enrolled**	**Date completed**	**Status (Active/ completed / Withdrawn)**	**Reason of withdrawal**	**Adherence**	**Intervention duration**
1						
2						
3						
4						

Adverse events (AE) Patient ID

Patient ID	AE Onset	AE End	Severity	Related to intervention	Action taken	Outcome	Comment
1							
2							
3							
4							

Appendix

Withdrawal Form

I. ..... myself thesis case no..... of Dr. Md. Rezwanul Haque Hereby I withdraw myself/ my patient from signed protocol of treatment. I will continue the treatment of myself/my patient according to standard protocol

Name of participant..... Signature/Thumb Impression:

Name of Witness:..... Signature/Thumb Impression:

Date: .....

Name & Signature of researcher.....