PROTOCOL

Title: To Evaluate the Efficacy of Probiotic – BIOTHERAPI ®
(Combination of Bacillus Subtilis 1972 and Bacillus Coagulans 1969) as an adjunctive therapy in the treatment of Rheumatoid Arthritis – A Prospective Study.

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1. Introduction:

RA is an autoimmune disorder in which unchecked immune and inflammatory responses cause articular pain and eventually cartilage degradation and bone destruction **(1)**. Disease develops when there is an imbalance in the cytokine network, either from excess production of pro-inflammatory cytokines or from inadequate natural anti-inflammatory mechanism.**(2)**

The concept that micro organisms play a role in etiology, pathogenesis, and treatment of RA has been advanced for over hundred years**(3)**. A more specific link between the microbiome and RA has been postulated on the basis of both animal data and basic science investigations of the microbiome of patients with the disease **(4)**. It is well know that periodontitis and RA share etiologic links to aging and smoking, as well as pathogenesis based in cytokine- mediated inflammation, tissue breakdown and bony erosions.**(5)**

The gut, too, can be a site of dysbiosis and, presumably, citrullination as well as other potential alterations that heighten the risk of developing RA and other auto immune diseases**(6)**. Studies in mice have shown that the gut microbiota is required for normal immune system maturation and development of tolerance. Should subsequent breaches in the epithelial barrier of the gut occur, a cascade of responses can result with a potential loss of tolerance. **(7)**

Gut organism can induce the expression of Ig A-Secreting B cells and Ig A can, inturn, regulate the composition of functional gut microbiota. Newly diagnosed RA patients, too, may have alterations in the predominant gut bacteria compared to healthy individuals, which may correlate with IL-17 production**(7)** .It has been shown that patients with Inflammatory arthritis have increase permeability of the gut due to inflammation of the gastro-intestinal tract.**(8)**

Probiotics are defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host **(9)**. It is not fully clear how probiotics work, but there is good evidence that they reduce gut permeability and modulate immunity. This modulation may come via increasing local secretory IgA immune responses to pathogens, reducing overgrowth of pathogenic bacteria, or down-regulating inflammatory immune factors such as TNF-α **(10)**. The use of probiotics to prevent or treat arthritis is quite unexplored but some studies have indicated the potential benefit. Combination treatment of Methotrexate and Probiotics (*Enterococcus faecium)* has also shown a potentiating effect, reducing clinical parameters compared to animals who receive only the methotrexate treatment. **(11)**

David, Mandal etal performed randomized,double blind, placebo controlled, parallel design clinical pilot trial to evaluate the effects of the lactic acid bacillus(LAB) Probiotc preparation, B.Coagulans, GBI-30, 6086 on symptoms and measures of functional capacity in patients with RA in combination with pharmacological anti-arthritic medications. Compared with placebo *B.Coagulans GBI-30 , 6086* treatment resulted in greater improvement in patients global assessment and self assessed disability, reduction in CRP , as well as ability to walk 2 miles, reach., and participate in daily activites.There were no treatment related AE reported throughout the study. It was concluded that large scale; completed clinical trials are needed to confirm these results. **(12)**

LAB have been shown to significantly downregulate pro-inflammatory cytokines (eg, IFN-γ, IL-12, TNF-α) without altering regulatory cytokines (eg, IL-10, TGF-β) to cause anti-inflammatory effects that alleviate RA symptoms. Therefore, it might be speculated that therapeutic strategies that aim to normalize the gut microflora in order to maintain proper gastrointestinal and immune system function may downregulate the abnormal inflammatory response and alleviate symptoms of RA. **(12)**

2. AIM of the Study:

The purpose of the study is to evaluate the effects of BIOTHERAPI-® **(**Combination of Bacillus Subtilis 1972 and Bacillus Coagulans 1969) on disease activity and functional ability of RA patients when used in combination with pharmacological anti-rheumatic medications.

3. Material and Methods:

Design: A Prospective, Randomized, Single centre, Two arm, Open label trial to evaluate the efficacy of Probiotic supplement to standard of care vs. standard of care alone in patients with Rheumatoid Arthritis.

Study Population: Subjects will be recruited from Department of Rheumatology, Yashoda Hospital Secunderabad.

Inclusion Criteria:

1. Are between the age of 18 to 75
2. Are clinically diagnosed with RA (according to ACR 2010 criteria) **(13)**
3. Have active disease defined by DAS 28 ESR>2.6
4. Should be on RA treatment for atleast 3 months and are expected to stay on stable dose throughout the study duration (i.e ongoing medication and/or other therapy such as physiotherapy are permitted, except immunotherapy).

ACR (2010) Classification Criteria for RA

Symptom Duration (as reported by patient)	Points
< 6 weeks	0
> 6 weeks	1

Joint Distribution	Points
1 large joint	0
2-10 large joints	1
1-3 small joints (with or without involvement of large joints)	2
4-10 small joints (with or without involvement of large joints)	3
> 10 joints (at least 1 small joint)	5

Serology	Points
RF- and CCP-	0
Low RF+ or CCP+	2
High RF+ or CCP+	3

Acute Phase Reactants	Points
Normal ESR or CRP	0
Abnormal ESR or CRP	1

RF: rheumatoid factor. CCP: anti-citrullinated citric peptide. ESR: erythrocyte sedimentation rate. CRP: C-reactive protein. Low: < 3 x upper limit of normal (ULN). High: > 3 x ULN

Requirements: patients who have at least 1 swollen joint, and not better explained by another disease to be applied. A score ≥ 6 points is required for classification as definite RA.

Exclusion Criteria:

Subjects will be excluded if they have

1. Chronic renal failure /renal tubular acidosis.
2. Pancreatitis.
3. Inflammatory bowel disease or leaky gut.
4. Currently consuming Probiotics with refusal to have a 2 week washout period, known to have allergies to the study product.
5. Planned to have a surgery during the time of the study.
6. Mental illness impairing ability to comply with study.
7. Women who are pregnant or plan to get pregnant during the study period, women who are breastfeeding
8. Any illness that could impair their ability to comply with the study, or were enrolled in another study
9. Exposure to > 10mg /day of Prednisolone
10. Plan to start with biological agents.
11. Subjects having arthritis other than Rheumatoid.

Treatment Arms :

Patients will be divided in two groups:
Group A: Probiotic Supplement + Standard of care treatment (Methotrexate, Hydroxychlroquine, Sulphasalazine, Leflunomide and steroids).

Group B: Only Standard of care treatment (Methotrexate, Hydroxychlroquine, Sulphasalazine, Leflunomide and steroids).

Randomization:

Alternate patient of RA will be given Probiotic supplements by the investigator free of cost provided by the **SANZYME** Company. Subject has to read the letter of information for the study and provide signed consent. Subjects will be randomized to receive one capsule orally, twice daily containing Probiotic supplements.

Interventions:
The active agent in this study is a Probiotic capsule containing Bacillus Subtilis 1972 and Bacillus Coagulans 1969, provided by SANZYME (P) Ltd. Each capsule contains not less than 5 billion Colony-Forming Unit (CFU).

Bacillus Subtilis 1972 is a gram positive non pathogenic bacteria which is commonly found in soil. It is considered as an ideal Probiotic as it also is a gut commensal in humans and has the ability to tolerate extreme environmental conditions. It is considered a tough Probiotic because it can form a protective endospore to keep itself alive almost indefinitely. It is stable at extreme PH levels and high temperatures, can survive desiccation as well as challenging storage/manufacturing conditions, and the gastric environment in the stomach. It is known for its ability to produce B-vitamins and is highly enzymatic in nature. It is known to modulate Immune system.

Bacillus Coagulans1969 is a gram positive non pathogenic bacteria derived from green malt. It is a widely used, well documented, safe and stable spore forming bacteria that promotes gastrointestinal health. It is widely used in food products and has high tolerance to thermal stability. It survives gastric acid barrier and bile, proliferates in intestine, Produces L+ lactic acid beneficial to gut and confers immunity, fights pathogens and supports a healthy gut. It is Generally Regarded As Safe (GRAS) product.

Blood Samples: Erythrocyte Sedimentation Rate (ESR), Serum Creatinine with eGFR, Complete haemogram, Aspartate Aminotransferase (AST or SGOT) and Alanine Aminotransferase (ALT or SGPT) and Complete Urine Examination.

Scheduled Visits: At day 0 (visit #1) (when recruited and just prior to starting to take capsules), at day 45±3 (visit #2), and at day 90± 3(visit #3), blood samples as a part of regular follow up will be collected by clinical technicians working in Yashoda Hospitals, Secunderabad.

At day 0, 45 and 90 (visits 1, 2 and 3) a physical exam will be completed, and PI will complete the Physician Global Assessment of Disease activity. In addition, at these times, the patients will be asked to complete the following self-administered questionnaire:

- Health Assessment Questionnaire (HAQ)

The Health assessment questionnaire disability index (HAQ-DI) is a questionnaire for the assessment of Rheumatoid Arthritis. The questionnaire is a patient reported outcome (PRO) which is usually self-administered by the patient.

The following categories are assessed by the HAQ:

1. dressing and grooming
2. arising
3. eating
4. walking
5. hygiene
6. reach
7. grip
8. common daily activities

DAS28 is a measure of disease activity in rheumatoid arthritis (RA). DAS stands for 'Disease Activity Score' and the number 28 refers to the 28 joints that are examined in this assessment**.**

To calculate DAS28-ESR:-

1. Count the number of swollen joints (out of the 28),
2. Count the number of tender joints (out of the 28),
3. Take blood to measure the erythrocyte sedimentation rate (ESR)
4. Patient to make a 'global assessment of health' (indicated by marking a 10 cm line between very good and very bad).

DAS28 ESR = 0.56 * sqrt (tender28) + 0.28 * sqrt (swollen28) + 0.70 * ln (ESR) + 0.014 * GH

In visit 2 and 3 the physician will confirm if the participant is taking the study capsule correctly, review any new medications the patient has taken since the last visit and will ask about adverse events that may have occurred and the count of capsule will be done by research coordinator at the second and third visit to evaluate compliance.

At day 90, subjects will stop taking capsules.

If a patient is withdrawn or withdraws from the study prematurely, he/she will be asked to receive a follow-up telephone call 30 days from the date they last took the study product. During the phone call, which should not take more than 10 minutes, the participant will be asked about any medications taken in the past month and about any possible side effects (unwanted effects or health problems) that they may have experienced.

Statistical Methods: The Primary outcome is to see the number of patients who shows improvement in DAS28-ESR score. Secondary outcome is to see the changes in HAQ and physician global assessment. Student t test shall be applied to see the difference between these two groups

Sample size: With a standard deviation of 0.7 for DAS28, effect size of 0.28, drop out of 15%, we would require 226 patients, to reject the null hypothesis with a rejection margin of 5% and power of 80%.

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