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# The Effect of *Lactobacillus reuteri* Supplementation on T-Regulator mRNA Gene Expression in Atopy Bronchial Asthma Adult Patient

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## Abstract

**Aim**: To understand the T-regulator mRNA gene expression changes after *L. reuteri* supplementation.

**Methods**: This research is using experimental research method, conducted in adult patients with atopy bronchial asthma that divided into two groups. The first group was supplemented with *L. reuteri* and the second group was given placebo. After two weeks, laboratory parameter was re-tested to assess T-regulator mRNA gene expression.

**Result**: There was a significant increase in T-regulator mRNA gene expression after *L. reuteri* supplementation (p<0,001) and no significant changes in control group (p=0.202). The velocity of T-regulator mRNA gene expression was 38.7%.

**Conclusion**: The increased T-regulator mRNA gene expression in *L. reuteri* supplemented group reflected the effectiveness of *L. reuteri* supplementation as therapy in adult patients with atopy bronchial asthma.

**Keywords**: Atopy bronchial asthma adult patient; T-regulator mRNA gene; *L. reuteri*

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## Introduction

Asthma is a chronic inflammation disorder in respiratory tract. Chronic inflammation caused hyper-responsiveness airway and manifested as episodic wheezing, shortness of breath, and coughing especially in the night or at early in the morning. Episodic symptoms were associated with degree of airway obstruction and usually reversible with or without treatment [1-3]. Inhaled corticosteroid is an effective drug to control and improve lung function, however high dose and long term usage are associated with increased side effects [4]. In fact, current asthma therapy had some limitations such as high cost therapy and some patients unresponsive with current therapy; therefore there is a need for better asthma therapy in term of effectiveness, safety, and as long term curative therapy or modifying disease course [5]. Probiotic is defined as non pathogenic, live microorganism that if consumed in certain amount will give benefit to the host. (FAO/WHO) [6-7]. *Lactobacillus reuteri* (*L. reuteri*) is a probiotic that had potential as anti inflammation, some studies conducted in human and animals showed positive result. *L. reuteri* in animal study showed potential in inducing Interleukin-10 (IL-10) to stimulate T-regulator (T-reg) cell, stimulating dendritic cell function, and changes immunological responses from T helper 2 (Th2) toward to Th1 [8-9].

## Methods

This study is a quasi-experimental study with double blind method. This study was conducted in Siloam Hospitals Manado. This study was carried out in one month, on September 2019. All subjects were observed for 2 weeks after intervention. This study included all intermittent and mild persistent asthmatic patients that had diagnosed for at least one year according to GINA criteria aged 16-51 years old. Patients must have stable condition for the last 4 weeks and not using oral or parenteral or inhaled corticosteroid in the past 2 weeks. Subjects were non smokers or smoke quitter for at least 1 year. Subjects must not consume probiotic in the past 2 weeks and agree to

**Table 1:** Characteristic and laboratory finding of patients.

| N=20 | Mean ± Standard deviation |
|---|---|
| Age, years old | 32.45 ± 8.22 |
| Ureum, mg/dl | 20.65 ± 5.26 |
| Creatinin, mg/dl | 0.89 ± 0.31 |
| AST, IU/ml | 17.9 ± 4.25 |
| ALT, IU/ml | 19.65 ± 9.37 |
| Random blood sugar, mg/dl | 101.85 ± 20.31 |
| Eosinophil, IU/ml | 385.35 ± 116.96 |
| IgE, IU/ml | 389.77 ± 432.05 |

**Table 2:** Comparison of T Reg mRNA Gene Expression between two groups.

| | L. reuteri group | Placebo Group | p Value | t Value |
|---|---|---|---|---|
| T Reg mRNA Gene Expression, pre intervention, Mean ± SD | 9.18 ± 0.98 | 8.89 ± 0.68 | 0.23 | 0.755 |
| T Reg mRNA Gene Expression, post intervention, Mean ± SD | 12.67 ± 0.91 | 8.99 ± 0.47 | <0.001 | 11.37 |

not consume probiotic contained food or drink. Exclusion criteria in this study were patients with active or past tuberculosis, had history for BCG vaccination, currently undergone specific immunotherapy with house mitt, HIV and AIDS infection, malignancy, malnutrition (body mass index <18.5 kg/m$^2$), diabetes, chronic kidney disease, sepsis, pregnant and lactating woman, patients using hormonal contraception, and mental disorder.

Eligible patients were divided into two groups, interventional and placebo group. Both groups will be checked for liver and kidney function test, random blood sugar level, eosinophil, Immunoglobulin E (IgE), Treg gene expression plasma level. Patients in interventional group will be supplemented with tablet of *L. reuteri* (Interlac®) containing $10^8$ CFU three times daily for two weeks. Monitoring was performed every two weeks to assess possible side effects occurrence. Laboratory test for mRNA Treg gene expression will be rechecked after 2 weeks of intervention using real time PCR.

The expression of mRNA Treg gene examination was carried out in Molecular Biology and Microbiology Laboratory Faculty of Medicine, Hasanuddin University, Makassar, Indonesia. All data was processed using Statistical Program for Social Sciences (Version 23: Armonk; NY: IBM Corp). Normality test of data distribution was tested using Kolmogorov Smirnov method.

## Results

During study period, 20 eligible patients were included for final analysis. They were divided into two groups for each 10 patients. Eligible subjects had mean age of 32.45 years ± 8.22 years old, mean eosinophil serum level of 385.35 IU/ml ± 116.96 IU/ml, and high IgE serum level of 389.77 IU/ml  432.05 IU/ml (Table 1). We found no significant difference between *L. reuteri* group and placebo group (p=0.23) before intervention. After 2 weeks of *L. reuteri* supplementation, the subjects in *L. reuteri* group had significant higher level of Treg mRNA gene expression compared to placebo group (12.67 ± 0.91 *vs.* 8.99 ± 0.47, p=<0.001, t=11.37) (Table 2). Significant increase of Treg mRNA Gene Expression was found in *L. reuteri* group from 9.18 ± 0.98 to 12.67 ± 0.91 (p=0.001). While in placebo group, there was no significant increase of Treg mRNA Gene Expression (Table 3). Further analysis showed velocity changes as much as 38%.

**Table 3:** Changes in T Reg mRNA Gene Expression level after intervention.

| | Pre | Post | p Value |
|---|---|---|---|
| T Reg mRNA Gene Expression, *L. reuteri* group | 9.18 ± 0.98 | 12.67 ± 0.91 | 0.001 |
| T Reg[10] mRNA Gene Expression, Placebo group | 8.89 ± 0.68 | 8.99 ± 0.47 | 0.202 |

## Discussion

Asthma is a disease characterized with bronchus hyperreactivity. Specific stimulus may induce airway narrowing in variable degree. Airway narrowing was associated with smooth muscle hyperreactivity and inflammation such as mucus hypersecretion, airway lining edema, epithelal desquamation and infiltration of inflammation cell [10]. Chronic inflammation of airway was the main pathogenesis of asthma bronchial, with increased granulated mast cell, eosinophil infiltration, and activated Th-2 cell [11]. Current management of asthma was based on controller and reliever therapy [12]. Controller is drugs that are used to prevent asthma exacerbation and one of the main controller therapies is corticosteroid. Corticosteroid is used to diminished the inflammation state on the airway, however long term use of corticosteroid raised concerns regarding the side effects [11,13].

Probiotic supplementation was proposed as adjuvant therapy for asthma bronchial. The rationale was in some studies, probiotic had anti inflammation effect that may alter the disease course of asthma. In case of asthma bronchial alteration of Treg level may decreased the chronic inflammation degree, due to effect of Treg in suppressing T lymphocyte proliferation, especially Th2. Suppression of Th2 effect by Treg may explained by the direct effect of Treg in suppressing CD4 or by suppressing antigen presenting cell function therefore lowering activation of T lymphocyte [14]. In this study we found significant increase of Treg mRNA gene expression after *L. reuteri* supplementation. This finding may reflect the ability of *L. reuteri* in altering Treg response and may help in asthma treatment. Previous study by Forsythe et al. found that oral supplementation of *L. reuteri* may diminished asthmatic inflammation responses in rats, which reflected by decreased eosinophil influx into airway lumen and parenchyma, decreased TNF level, monocyte chemoattractant protein-1, IL-5 dan IL-13 in bronchoalveolar lavage fluid. Study Forsythe give insight in mechanism of *L. reuteri* decreasing allergen induced airway hyperresponsiveness. This response was related with TLR-9 and increased activity of indoleamine 2,3-dioxygenase [15]. Our previous study was focused in the clinical response of *L. reuteri* supplementation for adult patients with atopy bronchial asthma. We found that *L. reuteri* supplementation may give clinical improvement which shown by significant improvement in clinical score, drug score, and Peak Expiratory Flow Rates (PEFR). Study by Yu et al. [16] using rats as subjects; also found that the use of *Lactobacillus rhamnosus* was associated with decrease of airway hyperresponsiveness, total eosinophile count, and IgE total level. Another study by Tubelius, who gave $10^8$ CFU of *L. reuteri* for 80 days to healthy worker, showed that sick leaves were more common in placebo group (26.4% *vs.* 10.6%, p<0.01) and also total days of not working (0.9% in placebo group *vs.* tr 0.4% in *L. reuteri* group, p>0.01) [17]. Chen et al. were conducting study in children aged 6 to 12 years with asthma and rhinitis allergy. They gave *L. gasseri* for 2 months and they found significant improvement in *PEFR,* decreasing clinical score, TNF-α, IFN-α, IL-12 dan IL-13 [18]. Lee at el. [19] found that the use of kefir in rats may lower TH2 cytokine product such as IgE, Il-4, and IL-13.

However some studies found that the use of probiotics may not giving significant effect for asthma condition. Giovanni et al found that supplementation of *L. casei* for 12 months in asthmatic children aged 2 to 5 years old may not giving significant improvement [20]. Kukkonen et al. [21] also found that probiotic supplementation was not associated with decreased in cumulative incidence for allergic disease, but may lower IgE associated (atopic disease) [21]. Rose et al. [22] also found that there was no significant changes for IgE, IgE specific, eosinophil and TGF beta after supplementation *L. rhamanosus* for 6 months in children aged 6 to 24 years old [22]. In this study, we present evidence that *L. reuteri* supplementation for adult asthma patient may give benefit which reflected with increased of Treg mRNA gene expression, where many published studies are focused in children population. The limitation of our study was Treg soluble plasma level was not checked therefore we cannot conclude whether Treg mRNA Gene expression as associated with increased Treg soluble plasma level.

## Conclusion

Probiotic supplementation especially *L. reuteri* may benefit for asthma therapy, which reflected with increased Treg mRNA gene expression that may associated with decreased chronic inflammation state of asthma patient. Further studies are warranted to make specific recommendation regarding the use of probiotic as adjuvant therapy for adult asthma patient.

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