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ORIGINAL ARTICE

Correlation Between Gut Microbiota Composition and Allergic Rhinitis Among School-aged Patients

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ABSTRACT

Background: During the past decades, prevalence of allergic diseases such as allergic rhinitis, bronchial asthma is rapidly increasing in developed countries.
Methods: This case control study was carried out on 52 school-aged patients who were classified into: allergic rhinitis group: 26 individuals, and Control group: 26 individuals without symptoms of allergic rhinitis. Eosinophilic count by blood cell counter was done. We used SYBR Green real time PCR by extraction of RNA according to RNeasy Mini Kit instructions for identifying bacteria (lactobacillus and bacteroides).
Results: There is significant difference between cases and control regarding lactic acid bacteria RNA and bacteroides bacteria RNA as the 1st type was significantly higher among the control group compared to the cases group while the 2nd type was significantly higher among the cases group compared to the control group.
Conclusion: The presence of bacteria of order lactic acid and bacteroid bacteria in gut microbiota of school-aged individuals may affect sensitization to different allergens.

Keywords: Gut Microbiota, Allergic Rhinitis, lactic acid bacteria RNA, bacteroides bacteria RNA status.

INTRODUCTION

In recent years, allergic diseases have been included by the WHO as one of the "major three diseases of the 21st century" and listed by the World Allergy Organization (WAO) as "a public health problem of global concern" [1].

This finding has been attributed to the hygiene hypothesis and changes in gut microbiota caused by eating habits and lifestyle. These microbiotas can be affected by several internal and external factors, such as diet, genetics, hormones, birth mode, pollutants, medication, and age [2].

Absence of early exposure to antibiotics, vaginal delivery, and exposure to pets during pregnancy are usually associated with lower rates of allergy [3,4].

During infancy, microbial exposure of the mucosa of airway triggers immunity development, the progressive colonization of the gastrointestinal tract enhances maturation of both the innate and adaptive immunity [5].

Increasing evidence strongly suggests that several allergy-protective effects arise via altering the gut microbiota in early childhood during the first year of life [6].

Previous reports have shown that the use of drugs that could directly cause intestinal dysbiosis, increased the risk for allergy

development via altering the human microbiome [7,8,9].

We have conducted this work to study the relationship between the gut microbiota types and allergic rhinitis.

METHODS

Written informed consent was obtained from all participants and the study was approved by the research ethical committee of Faculty of Medicine, Zagazig University. The study was done according to The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Study design: Our case control study was held on 52 school-aged patients presented at Oto-rhino-laryngology, Head and Neck (ORL-HNS) Department and Microbiology and Immunology department. This study was done between June and December 2020. The patients were classified into two groups each one includes 26 patients. The first one was **Allergic rhinitis group:** with allergic rhinitis, and second was **Control group:** without allergic rhinitis symptoms. **Inclusion criteria** for AR population were individuals age ranging from (6-18) years old, patients were selected according to the ARIA classification (Allergic Rhinitis and its impact on Asthma), with two or more of the following allergic rhinitis symptoms: (Sneezing'- Aqueous rhinorrhoea – 'especially paroxysmal" – nasal itching– nasal obstruction – conjunctivitis "itching-lacrimation or redness") and individuals presented with persistent or perennial type of allergic rhinitis, in which signs are present: (more than four days a week and for more than four consecutive weeks). **Exclusion criteria** for AR population were purulent rhinorrhoea, pregnant and lactating women, diabetic patients (glucose ≥ 126 mg/dl), BMI values > 30 kg/m^2, patients presented with dyslipidaemia (LDL cholesterol ≥ 189 mg/dL and/or triglycerides ≥ 350 mg/dL), patients with systolic blood pressure ≥ 160 mmHg and/or diastolic blood pressure ≥ 100 mmHg, patients receiving treatment with antibiotics 30 days before the start of the study, patients receiving treatment with corticosteroids 30 days before the start of the study or individuals who usually intake prebiotics and/or probiotics supplements 30 days before the start of the study according to **Okubo et al., 2017** [10].

Laboratory procedure: Eosinophilic count by blood cell counter (Sysmex) was done and Skin prick tests (SPT) as well for common perennial and seasonal allergens to confirm or exclude allergic rhinitis in patients and control groups respectively through SPT by immersing the lancet, which is accurately titrated, in the diagnostic vial then applying it to the skin of the front of left forearm inside a drawn circle for 30 seconds. This was also applied for +ve & -ve controls. All tests were read after 20 minutes and the result was considered positive **(Figure 1)** when the resulting wheals diameter or flaring, measured by a tape, was 3mm or more or 2mm more than the negative control.

We used SYBR Green real time PCR for identifying bacteria (lactobacillus and bacteroides) by extraction of RNA According to RNeasy Mini Kit instructions. Analysis of the SYBR green rt-PCR results was done. Amplification curves and Ct values were determined by the stratagene MX3005P software. To estimate the variation of gene expression on the RNA of the different samples, the Ct of each sample was compared with that of the control group according to the "ΔΔCt" method stated by **Yuan et al**. [11]. Dissociation curves were compared between different samples to exclude false positive results. Whereas ΔΔCt = ΔCt reference (Housekeeping gene) – ΔCt target. ΔCt target gene = average Ct of control – Ct of each allergic patient and ΔCt reference (Housekeeping gene) = Ct control - Ct of each allergic patient.

Statistical analysis: Statistical analyses were performed using IBM SPSS 22.0. A comparative statistical analysis of all variables was carried out using Student's t test and Fisher's exact test. The level of significance was set at $p < 0.05$ for both tests.

RESULTS

Both groups were similar regarding sex distribution, age, and BMI but regarding family history it was significantly associated with cases as reported in detail in **(Table 1).** Mean disease

duration was 6.15±2.01 and regarding classification the majority were intermittent and mild. Also, the majority were triggered by inhalation **(Table 2)**. Majority of cases showed house dust and date palm pollens allergy.

Absolute eosinophilic count was significantly higher among cases (0.66±0.11) compared to controls (0.21±0.071) (p <0.001). Lactic acid bacteria RNA was significantly higher among control group (0.98±0.06) compared to cases group (0.21±0.10) while bacteroid bacteria RNA was significantly higher among cases group (11.07±1.84) compared to control group (0.95±0.08) (p <0.001).

There was a statistically significant –ve correlation between fold change of Lactic acid and bacteroides among cases group **(Figure 2)**.

There was a statistically significant decrease in lactic acid bacteria fold change among cases triggered by both food and inhalation compared to cases triggered by food only or inhalation only. Also, there was a statistically significant increase in bacteroid bacteria fold change among cases triggered by both food and inhalation compared to cases triggered by food only or inhalation only **(Table 2)**.

Table 1: Demographic data distribution among the studied groups.

Variable			Case (n=26)	Control (n=26)	t/ χ2	P
Age (years) mean ± Sd			**11.19±3.26**	**11.65±3.24**	**0.511**	**0.612 NS**
BMI (Kg/m^2) mean ± Sd			**23.15±2.54**	**23.73±2.16**	**0.897**	**0.374 NS**
Sex	**Male**	**N**	**12**	**14**	**0.308**	**0.57 NS**
		%	**46.2%**	**53.8%**		
	Female	**N**	**14**	**12**		
		%	**53.8%**	**46.2%**		
FH	**-VE**	**N**	**13**	**24**	**11.33**	**0.001***
		%	**50.0%**	**92.3%**		
	+VE	**N**	**13**	**2**		
		%	**50.0%**	**7.7%**		

Sd: Standard deviation, t: Independent t test, χ2: Chi square test, NS: non-significant (P>0.05)
*: Significant (P<0.05)

Table 2: Relation between fold change of lactic acid bacteria/ld change of bacteroides bacteria and (sex, family history and diseases data) among cases group.

Variable		N	lactic acid	t/ F	P	Bacteroides	t/ F	P
Sex	**Male**	12	0.22	0.67	0.51 NS	10.95	0.29	0.77 NS
	Female	14	0.19			11.17		
FH	**-VE**	13	0.19	1.19	0.25 NS	11.34	0.73	0.47 NS
	+VE	13	0.24			10.80		
Type	**Intermittent**	18	0.22	0.87	0.39 NS	11	0.28	0.78 NS
	Persistent	8	0.18			11.23		
Severity	**Mild**	16	0.20	0.62	0.54 NS	11.08	0.03	0.98 NS
	Moderate to sever	10	0.23			11.06		
Triggers	**Inhalation**	**13**	**0.23**	**3.51**	**0.04***	**10.3**	**13.6**	**<0.001 ****
	Food	**5**	**0.26**			**9.91**		
	Both	**8**	**0.14***			**13.05****		

Sd: Standard deviation, t: Independent t test, F: ANOVA test, NS: non-significant (P>0.05)
*: Significant (P<0.05)

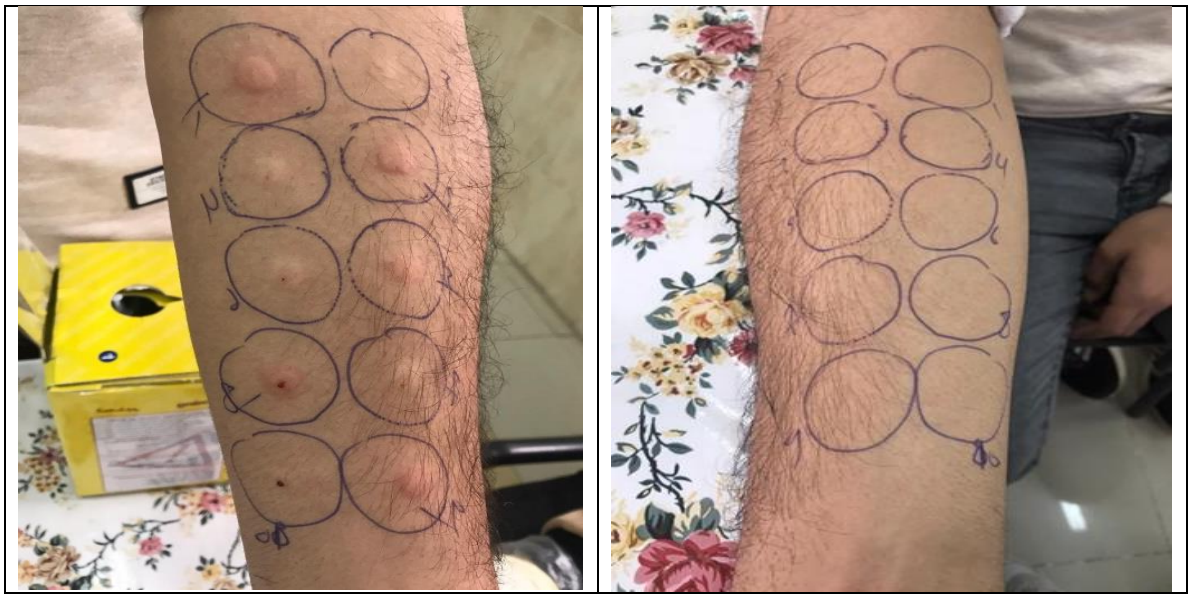

Figure 1: Example of Skin Prick Test in this study; each number is code of an allergen.

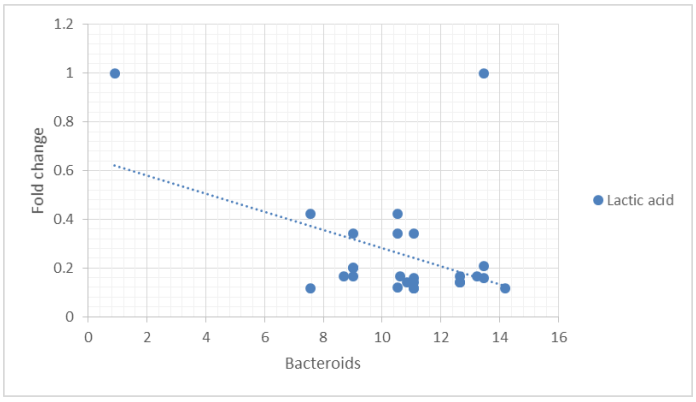

Figure 2: Correlations between fold change of Lactic acid and bacteroids among cases group.

DISCUSSION

Most children suffer from allergic airway problems during childhood [12,13]. Many factors in the early life affect the development of allergic rhinitis in genetically susceptible subjects. The intestinal microbiota plays a major role in the development and regulation of the immune system, and its composition is associated with the development of several immune-mediated and non–immune-mediated diseases [14].

In this study we aimed to study the gut microbiota in patients with allergic rhinitis (case group), compared with people without allergic rhinitis (control group). In the current study, there was no significant difference between cases and control regarding age, gender, and family history. This came in agreement with Arrieta et al., [15] who found that there was no significant difference between cases and control regarding gender. Also, Wafaa [6] and Chiu et al., [12] found that there was no significant difference between cases and control regarding gender, age, and family history.

In the present study, 50% of patients had family history of allergic rhinitis. This came in agreement with Saleem et al., [17] who found that a positive family history of allergic diseases was found in 56.9% of patients. In the present study, there was a significant difference between the studied groups regarding eosinophils which were higher in AR group than control group. This came in agreement with Chen et al., [18] who found that patients with AR have elevated

levels of eosinophils. In the present study, incidence of sensitization to any allergen was 100% and majority of allergens were house dust and date palm pollens. This came in agreement with Chiu et al., [19] who found that mite-test appears to be significant in childhood allergic rhinitis.

Saleem et al., [17] found that the overall rate of sensitization to any allergen was around 90%, with 10% of the patients were negative. In the current study, out of all patients, 69.2% were poly-sensitized [positive skin reaction to at least two allergens]. This came in agreement with Saleem et al., [17] who found that out of all patients, 78% were poly-sensitized.

In the current study, most common allergen was plant pollen and the most common found was date palm pollens. This came in agreement with Saleem et al., [17] who found the same results. Also, Wafaa and Ghada [20] found that mite and date palm pollens are the most common allergens.

In the present study, RNA of both lactic acid and bactericides were different significantly between cases and controls with statistically significant decrease in lactic acid bacteria among cases triggered by both food and inhalation compared to cases triggered by food only or inhalation only and a statistically significant increase in bacteroides among cases triggered by both food and inhalation compared to cases triggered by food only or inhalation only. In agreement with our study Ling et al. [21] stated that Bacteroidetes were lesser and Firmicutes [including Clostridiaciae] were higher in 5-month-old food-allergic infants ensuring that these insults might preclude the disease development. Also, a prospective study by Nylund et al. [22] found lower abundance of Bacteroidetes and greater abundance of Clostridium clusters IV and XIVa at 18 months in children who are diagnosed later with eczema.

Arrieta et al. [15] explored the relationship between gut bacterial and fungal dysbiosis in children and the development of atopic wheeze in later childhood in a representative sample of an Ecuadorian birth cohort [ECUAVIDA], which has studied peoples living in a rural region in a tropical non-industrialized country. This represents a distinct environment from all previous studies of the effects of the microbiome on asthma development done in developed settings.

Abrahamsson et al. [23] and Bisgaard et al. [24] have concluded that altered microbial variation in early infancy causes the development of allergic rhinitis and asthma at school age children and gut microbiota co-evolves with the infants' immune system. Abrahamsson et al. [25] performed a Swedish cohort study on 40 infants and they found a relation between atopic eczema at 2 years with a lower diversity of Bacteroidetes at 1 month of age.

In the KOALA birth cohort of nearly 1,000 infants, colonization with C difficile at 1 month of age was associated with high risk of recurrent wheeze, allergic sensitization, eczema, and asthma by 7 years of age [27]. Vael et al. [26] found that colonization with Clostridium coccoides subcluster XIVa also has been associated with an increased risk of asthma at 3 years of age.

Microbiota has well established role in health and food allergy with reference to the human gut [28]. Smaller proportions of Bacteroidetes and larger proportions of Firmicutes have been found to be associated with food-allergy in infants [21]. However, sensitization to aeroallergens occurs after infancy, specially, in the development of rhinitis and asthma in children [19].

To date, no specific bacterial taxa have been consistently associated with allergic disease or other conditions. Some studies have suggested that early changes of gut microbiota might be more important than the presence or absence of specific taxa [29,30,31,32].

The intestinal microbiome differs in different studies, this may be due to geographic location, which is likely explained by differences in lifestyle, diet, and environmental exposures [33].

Limitations: The current study has limitations, for example, the number of participants was not quietly enough to judge the technique.

Conclusion: The presence of bacteria of order lactic acid and bacteroid bacteria in gut microbiota of school-aged children may affect sensitization to different allergens. However, heterogeneity in study design, including sampling time points, methods used to characterize microbiota, and different allergic phenotypes under study, make it difficult to establish a causal relation between specific bacterial taxa and development of allergy.

CONFLICT OF INTEREST
The authors declare no conflict.
FUNDING
Not applicable

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