Original Article

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Changes in *Lactobacillus* and *Bifidobacterium* Load in a Population of Iranian People with Papillary Thyroid Cancer: The Effect of Radioactive Iodine therapy

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Abstract

Background: To investigate the changes in *Lactobacillus* and *Bifidobacterium* load in a population of Iranian people with Papillary Thyroid Carcinoma (PTC) before and following Radioactive Iodine (RAI) therapy.

Methods: This longitudinal cohort study included 28 healthy individuals and 30 PTC patients referred for RAI therapy. A total of 90 stool samples from the same 30 patients with PTC were collected one day before RAI therapy, two weeks and two months following RAI therapy. As a control group, 28 stool samples were collected from 28 healthy age-sex matched individuals. Stool microbiotas were detected using 16S rRNA gene sequencing.

Results: *Lactobacillus* load was significantly lower in patients with PTC compared to the healthy individuals before (2.56 *vs.* 4.47 copies/*gr* stool, p=0.01) and two weeks after RAI therapy (3.51 *vs.* 4.47 copies/*gr* stool, p=0.02). Two months after RAI therapy, there was no significant difference in the load of *Lactobacillus* between the two groups (5.21 *vs.* 4.47 copies/*gr* stool, p=0.69). Moreover, the load of *Lactobacillus* increased significantly from 2.42 copies/*gr* stool before RAI therapy to 3.80 copies/*gr* stool at two weeks, and 5.27 copies/*gr* stool after two months of RAI therapy (p=0.01). On the other hand, there was no difference in the abundance of *Bifidobacterium* between the PTC and healthy individuals at different time points.

Conclusion: This study indicated the difference in the load of *Lactobacillus* in PTC patients compared to healthy individuals. Moreover, it highlighted a significant increase in the load of *Lactobacillus* in PTC patients following RAI therapy. These results highlight the potential for microbiota modulations as an adjuvant therapy in PTC management

Keywords: Gut microbiota, Radioactive iodine, Thyroid carcinoma

Introduction

Gut microbiota have been known to have a complex interaction with both the development and treatment of different type of cancers (1). Recent studies have highlighted the significant role of gut microbiota in the modulation of immune responses and its potential influence on the development and progression of Papillary Thyroid Cancer (PTC) (2,3). The gut microbiome can also impact the efficacy of iodine therapy and may alter the tumor environment, affecting tumor growth and metastasis (4,5).

On the other hand, iodine therapy, particularly in the context of Radioactive Iodine (RAI) treatment for patients with PTC, has been shown to influence gut microbiota (6). Research suggests that iodine therapy through the alteration in thyroid function can lead to changes in the microbial composition of gut. This shift may be attributed to the potential impact of thyroid hormones on gut motility and mucosal immunity, which can affect microbial balance (7-9). Additionally, iodine treatment may contribute to an increase in certain bacterial populations while decreasing others, potentially resulting in dysbiosis (10). The relationship between thyroid hormones and gut microbiota is bidirectional and complex. Some studies suggest that hypothyroidism can cause changes in gut microbiota (11,12). Gut microbiota plays a crucial role in regulating the thyroid function as well. Composition of gut microbiota can affect the thyroid function through the alteration in the absorption of trace elements like selenium and iodine, impairment in the conversion of T4 to T3, and modulation of the immune system (13). Analyzing the composition and diversity of gut microbiota in patients with PTC, both before and after iodine therapy, could provide insights into how microbial populations influence cancer progression and treatment outcomes. Considering the potential role of *Lactobacillus* and *Bifidobacterium* in decreasing the risk of thyroid cancer, this study was conducted to evaluate the changes in *Lactobacillus* and *Bifidobacterium* loads in a population of Iranian people with PTC before and following RAI therapy, compared to healthy individuals.

Materials and Methods

Study participants and sample collection

This longitudinal cohort study included 28 healthy individuals and 30 PTC patients referred for RAI therapy. The inclusion criteria were: being candidate for RAI therapy, age less than 55 years, lack of known metastasis, not using antibiotics, probiotics, or prebiotics within two weeks before the initiation of RAI therapy, and two months after receiving RAI therapy. The two time points for collecting stool samples (at two weeks and two months after RAI therapy) were chosen based on half-life of RAI (8 days) and the time required for complete removal of RAI (approximately 32 days) (14). Informed consent was obtained from the participants; and the study protocol was approved by the ethical committee at Iran University of Medical Sciences (IR.IUMS.REC.1398.1413).

DNA extraction

200 *mg* of stool samples from both patients and healthy individuals were used to extract total metagenomics DNA using the QIAamp DNA stool Mini Kit (Qiagen, Retsch GmbH, Hannover, Germany), following the instructions provided in the kit manual. The quality of isolated DNA was evaluated using a Nanodrop spectrophotometer (MAESTRO-Gene). Isolated DNA was then stored at 4°*C* until further processing.

Primers used in quantitative real-time PCR (qPCR)

The specific primers for quantitative assays were based on the 16SrRNA sequence of *Lactobacillus* and *Bifidobacterium* species obtained from our previous study and those conducted by Matsuki *et al* (15,16), which are shown in table 1. The quantitative real-time PCR was performed in the ABI StepOne Plus™ real-time PCR instrument (Applied Biosystems, USA), as follows master mix reaction in total volume of 20 *µl*, which included 12.5 *µl* of Add SYBR Green master mix (Addbio, Korea), 1 *µM* for each primer in final concentration and 50 *ng* metagenomics DNA. The amplification program consisted of an initial denaturation for 10 *min* at 95*°C*, followed by 40 cycles of 15 *s* denaturation at 95*°C*, annealing at 60°*C* for 30 *s*, and extension at 72°*C* for 30 *s*.

Standard curve

The standard curve of the bacteria abundance against

Table 1. Primers used in Real-Time PCR to identify the desired bacteria

Target bacteria	Primer	Oligonucleotide sequences (5'-3')	Size (*bp*)	Product size (*bp*)
Bifidobacterium group	primer F	CTCCTGGAAACGGGTGG	17	549
	primer R	GGTGTTCTTCCCGATATCTACA	22	
Lactobacillus group	primer F	GTCTGATGTGAAAGCCTTCG	20	204
	primer R	CCAGGGTATCTAATCCTGTTCG	22	

Table 2. Lactobacillus count before and after Iodine therapy in PTC patients compared to healthy individuals*

Time point	PTC patients	Healthy individuals	p-value
Before Iodine therapy	3.20±2.44	4.71±2.13	0.023
Two weeks after Iodine therapy	3.12±1.97	4.71±2.13	0.009
Two months after Iodine therapy	4.79±2.88	4.71±2.13	0.906

* copies/gr of stool; PTC: Papillary Thyroid Cancer.

DNA concentration was prepared using 10 different standard strain concentrations (10^1 to 10^{10} CFU/*ml*). The qPCR was carried out in duplicate using extracted DNA from standard strain *Lactobacillus acidophilus* ATCC 4356 and *Bifidobacterium bifidum* ATCC 29521. The mean cycle threshold (Ct) values are used for the standard curve. The Ct value of extracted metagenomics DNA was compared with the standard curve that allowed us to determine the bacterial load (CFU/*ml*) in finally.

Statistical analysis

The data were analyzed using the IBM SPSS version 20.0 software (IBM Corp., Armonk, NY, USA) and all quantitative results are presented as mean ± SD. The distribution of data was assessed using the Shapiro–Wilk test, and based on the results, the student's t-test was used for the comparison of bacterial load between study groups at different timepoints. Additionally, repeated measures analysis of variance (ANOVA) test was used to analyze the changes in bacterial load in the patient group. Significant results were determined and reported based on p values equal to or smaller than 0.05.

Results

Clinical characteristics of the participants

A total of 90 stool samples from the same 30 patients with PTC were collected at one day before RAI therapy as well as two weeks and two months following RAI therapy. Moreover, 28 stool samples were collected from 28 healthy age-sex matched individuals. All participants were women. The mean age of the PTC patients was 39.13 (8.88) years and that of the healthy individuals was 35.85 (8.44) years (p=0.156). All the PTC patients received RAI with dose of 100 *mCi* (56%) or 150 *mCi* (44%).

Microbial load in PTC patients before and following RAI therapy compared to the healthy individuals

Before RAI therapy and at two weeks after RAI therapy, the abundance of *Lactobacillus* was significantly lower in PTC patients compared to the healthy individuals (3.20 *vs.* 4.71 copies/*gr* stool, p=0.023) and (3.12 *vs*. 4.71 copies/*gr* stool, p=0.009). However, there was no significant difference in the load of *Lactobacillus* between the two groups, at two months after RAI therapy (4.79 *vs.* 4.71 copies/*gr* stool, p=0.906) (Table 2).

However, there was no significant difference in the load of *Bifidobacterium* between PTC patients and healthy individuals before and at two time points after RAI therapy (Table 3).

Table 3. *Bifidobacterium* count before and after Iodine therapy in PTC patients compared to healthy individuals*

Time point	PTC patients	Healthy individuals	p-value
Before Iodine therapy	6.80±1.09	7.15±0.82	0.187
Two weeks after Iodine therapy	6.79±1.05	7.15±0.82	0.199
Two months after Iodine therapy	7.09±1.26	7.15±0.82	0.838

*copies/gr of stool; PTC: Papillary Thyroid Cancer.

Table 4. Changes in the load of Lactobacillus and Bifidobacterium in PTC patients following Iodine therapy

Genus	Before Iodine therapy	Two weeks after Iodine therapy	Two months after Iodine therapy	p-value
Lactobacillus count	2.42±2.09	3.80±2.00	5.27±2.73	0.014
Bifidobacterium count	6.62±1.12	6.68±1.17	7.38±1.22	0.085

*copies/*gr* of stool; PTC: Papillary Thyroid Cancer.

Alteration in microbial load in PTC patients before and following RAI therapy

Lactobacillus load increased significantly over the three time points, from 2.42 copies/*gr* stool before RAI therapy to 3.80 copies/*gr* stool at two weeks, and 5.27 copies/*gr* stool after RAI therapy (p=0.014). There was no significant change in the load of *Bifidobacterium* before RAI therapy and at the two time points after RAI therapy (Table 4).

Correlation between TSH levels and Lactobacillus/Bifidobacterium

Also the correlation between *Lactobacillus*/*Bifidobacterium* and TSH level was evaluated. However, there was no meaning-full correlation between TSH level and neither *Lactobacillus* nor *Bifidobacterium* before and after iodine therapy (Supplementary table 1).

Adverse events

Most GI-related symptoms observed post iodine therapy were nausea (40%), diarrhea (20%), dry mouth (20%), and heartburn (6.67%), and vomiting (3.3%). However, there was no meaning-full correlation with the microbiota change.

Discussion

Recent evidence suggests a relationship between gut microbiota, such as *Lactobacillus*, and thyroid health (17). Specific strains of *Lactobacillus* may have immunomodulatory effects, potentially influencing the microenvironment of tumors (18,19). For instance, a study by Lu *et al* found that certain probiotics, including *Lactobacillus*, can help regulate immune responses and may play a role in decreasing the risk of thyroid cancer by modulating inflammatory pathways and promoting gut health (18).

Confirming the results of previous studies, the current study indicated a significant lower load of *Lactobacillus* in PTC patients compared to the healthy individuals. This difference disappeared after RAI therapy. Likewise, another study found a significantly lower relative abundance of *Lactobacillus* in thyroid cancer compared to the healthy controls (10). A more recent study showed a significant negative correlation between the abundance of *Lactobacillus* and tumor volume (20). Moreover, they found a significant reduction in the relative abundances of *Lactobacillus* in tumor tissues with Lymph Node Metastasis (LNM) compared to those without LNM (20).

On the other hand, RAI therapy has been shown to change the gut microbiota (4,21). A recent *ex vivo* experiment indicated 3.46-fold increase in the abundance of *Lactobacillus* in fecal samples exposed to I-131 (21). Although the results of an experimental study cannot be directly compared with those of clinical study, the present study similarly showed an increase in the abundance of *Lactobacillus* following

Supplementary Table 1. Correlation between *Bifidobacterium* /*Lactobacillus* and TASH level

Bacteria	PTC Group	Status	Spearman's rho	p-value
Bifidobacterium	Before *vs.* after RIA	2 weeks *vs.* baseline	0.3333	0.2657
		2 months *vs.* baseline	0.3683	0.1004
		2 months *vs.* 2 weeks	0.0610	0.8099
Lactobacillus	Before *vs.* after RIA	2 weeks *vs.* baseline	0.4772	0.0721
		2 months *vs.* baseline	-0.1274	0.6783
		2 months *vs.* 2 weeks	-0.0021	0.9935

PTC: Papillary Thyroid Cancer.

RAI therapy. Another study also demonstrated different stages of RAI therapy exert various effects on gut microbiota (22). Moreover, there is also a bidirectional relationship between thyroid hormone level and microbacteria (R).

The alteration of microbiota following RAI therapy could be attributed to factors such as changes in dietary habits during treatment, the impact of radiation on gastrointestinal cells, and alteration in hormones due to thyroid ablation (22,23). These changes in the microbiota may affect patient outcomes, including recovery and quality of life, highlighting the need for further research to explore the mechanisms behind these associations and the potential for microbiota modulations as an adjuvant therapy in PTC management (4).

Bifidobacterium, another beneficial genus of gut microbiota, has been investigated for its potential influence on thyroid health and cancer (3,8). Evidence has shown that a balanced gut microbiome, including strains of *Bifidobacterium*, could help to prevent chronic inflammation and support overall immune function, potentially lowering the risk of thyroid cancer (8,24). However, direct studies on *Bifidobacterium* and PTC specifically are limited. The abundance of *Bifidobacterium* in PTC patients before and following RAT therapy was studied and compared to the healthy individuals. Nevertheless, no significant change was found in the *Bifidobacterium* load at different stages of RAI therapy between the two groups. Moreover, there was no significant change in the load of *Bifidobacterium* over the three timepoints.

Another study explored the alteration in the gut microbiota before and after the first and second episode of RAI therapy, found no significant difference in the abundance of *Bifidobacterium* before and after the second episode of RAI therapy, although *Bifidobacterium* was abundant after the first episode of RAI therapy (22). However, there is a complex interaction between gut microbiota, RAI therapy, and thyroid cancer. Although it is an evolving area of research, many factors (pollution, ethnicity, dietary habits, lifestyle, and drug intake) that exert a key role in shaping gastrointestinal microenvironment have not been taken into account in all studies.

Strengths and limitations

To our knowledge, this was the first study among a population of Iranian people to investigate alterations in the gut microbiota in patients with PTC at different time points following RAI therapy. This study included adequate number of PTC patients and healthy individuals for comparison. However, dietary habits and other environmental factors that may interact with gut microbiota were not taken into account in this study.

Conclusion

In conclusion, this study indicated a significant lower abundance of *Lactobacillus* in PTC patients compared to the healthy individuals; this difference disappeared following RAI therapy. Moreover, the load of *Lactobacillus* significantly increased over the time after RAI therapy in patients with PTC. However, this remains an observational association. We cannot

definitively state that RAI therapy caused the increase in *Lactobacillus*. The recovery over time could coincidentally be related to other post-treatment changes like improving in thyroid function.

While the result points to a potential beneficial side effect of RAI, further research is essential to confirm causality, elucidate the underlying mechanisms, and determine the clinical significance of this microbial shift.

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Ethics approval and consent to participate

This study was approved by the Ethics Committee at Iran University of Medical Sciences (Ethics Number: IR.IUMS.REC.1398.1413).

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Conflict of Interest

All authors declare that they have no competing interests.

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