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Dysbiosis questionnaire as a tool for an in-depth medical interview in the field of intestinal microbiota disorders in head and neck cancer patients

Original Study

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Abstract

Introduction. In-depth information on intestinal disorders and their contributing factors is rarely collected in the routine medical history of patients with head and neck diseases, in this number in head and neck cancer (HNC) patients. Intestinal dysbiosis (ID) is an important problem for patients starting HNSCC treatment, but there is no tool for a quick and unambiguous diagnosis and confirmation with objective tests. The aim is to design the tool for an in-depth medical interview in the field of intestinal microbiota disorders, namely the Dysbiosis Questionnaire.
Materials and Methods. A prospective study comparing 188 consecutive HNC patients and an age-matched group of 76 healthy individuals as to the frequency of intestinal ailments. The questionnaire was assessed for the frequency of individual responses and the variables with the highest degree of correlation with intestinal ailments were selected.
Results. While some lifestyle and medical factors, such as antibiotic use and physical activity, differ significantly among individuals with dysbiosis between HNC patients and the control group, other variables, including birth history, chronic diseases, and dietary habits, do not show statistically significant differences.
Conclusions. A mosaic of factors turns out to have an impact on the link with dysbiosis in HNC patients. The proposed Dysbiosis Questionnaire identifies these factors accurately and may be helpful in a quick selection of prone individuals.

Keywords

dysbiosis • head and neck cancer

1. Introduction

Gut microbiota (GM) serves key roles in the crosstalk between the intestinal and respiratory tracts, which is called the gut-lung axis, [1,2] via which gut microbe-derived agents modulate airway immunity [3-5]. Intestinal dysbiosis (ID) (i.e., a dysfunction of the GM) concerning both the amount and composition of the microbiota, gives multiple clinical symptoms.

The gut microbiome's role in cancer etiology has been a topic of many recent investigations [6]. Findings suggest that ID, which describes a general disturbance in the GM, underlies poorer outcomes in oncological patients. Additionally, oncological treatment uses an entire arsenal of therapeutic agents, which has an impact on GM. The burden of head and neck cancer (HNC) patients in terms of the development of ID is typical for this group: heavy smoking, the etiopathogenesis of cancers based on HPV infection, widespread use of antibiotics before cancer diagnosis when treating early symptoms [7,8], and then, as planned, perioperatively, the use of PPI, [9-11], RT or RT / CT [12-15].

The understanding of the human intestinal microbiome, primarily using sequence-based approaches, has developed greatly in recent years, nevertheless, the knowledge of HNC-GM interplay is rudimentary. In most clinical problems, clinicians ask questions that are answered by scientists from basic sciences, while research on the microbiota has an enormous molecular knowledge with the simultaneous lack of clinical analysis. The clinical translation must be incorporated into clinical practice because each NGS-based test will require a broadly defined financial outlay.

In-depth information on intestinal disorders and their contributing factors is rarely collected in the routine medical history of patients with head and neck diseases in this number in HNC patients. We set the hypothesis that ID is an important problem for patients starting HNSCC treatment, but there is no tool for a quick and unambiguous diagnosis and confirmation with objective tests. Thus, the tool for an in-depth medical interview in the field of intestinal microbiota disorders for HNSCC patients was designed and our goal is to validate the questionnaire in a prospective study comparing HNC patients and an age-matched group of healthy individuals. The first aim of our research is to analyze the questionnaire for the frequency of individual responses and to select the variables with the highest

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degree of correlation with intestinal ailments the second aim is to compare the studied and control population as to the frequency of intestinal ailments.

2. Materials and methods

A prospective study has been conducted on 188 consecutive HNC patients treated in Otolaryngology, Head Neck Surgery Poznan University tertiary referral Department between IX-XI 2021 and on an age-matched control group made up of 76 healthy individuals. All HNC patients who filled the Dysbiosis Questionnaire at admission were enrolled.

Demographic information including age, gender, and BMI was obtained during subject recruitment. An in-depth interview about intestinal complaints was taken with the Dysbiosis Questionnaire, which consists of closed and open questions concerning the socioeconomic data and medical data. The Dysbiosis Questionnaire is a proprietary tool, developed in our department after consulting a panel of internists in 2018.

The first part of the work is devoted to the analysis of the questionnaire, consisting of the assessment of the frequency of individual responses in both the control group and the studied population. Subsequently, the selection of variables/problems with the highest degree of correlation with intestinal ailments was indicated.

The second part of the work is devoted to comparing the frequency of intestinal ailments in the examined group and the control group.

The primary data, after evaluation of the results of laboratory tests, data from the history, and examination by a gastroenterologist, were the categorization of the patient by dysbiosis (yes or no). Variables included in the Dysbiosis Questionnaire: natural childbirth, breastfeeding, allergy, chronic diseases, medications, taking antibiotics, traveling, type of diet, playing sports, age, and gender were correlated with dysbiosis categorized as present-yes or absent-no.

Statistical analyses were conducted using a two-tailed proportion test to compare categorical variables between groups. Odds ratios (OR) with 95% confidence intervals (CI) were calculated to assess the strength of associations. The Mann–Whitney test was used for non-parametric comparisons, while the Chi–square test was used for categorical data. A significance level of $\alpha = 0.05$ was applied, and p-values below this threshold were considered statistically significant. Statistical analyses were conducted using Statistica 14.0 software.

3. Results

A total of 264 individuals participated in the study, including 188 patients with HNC (71.21%) and 76 individuals in the control group (28.79%). The median age of HNC patients was 60 years old, with the youngest patient being 24 years old and the oldest 82 years old. In the control group, the median age was 56 years, ranging from 40 to 83 years. No statistically significant difference in age was observed between the study and control groups (Mann–Whitney test, $p = 0.57503$). Among the HNC group, 76 participants were female (40.43%), while 112 were male (59.57%). In the control group, there were 28 females (36.84%) and 48 males (63.16%). No statistically significant difference in sex distribution was found between the HNC and control groups (Chi–square test, $p = 0.58953$).

Table 1. Prevalence of Dysbiosis in HNC patients and the Control Group

Group	Dysbiosis Yes	Dysbiosis No	Total	Prevalence (%)	95% CI
HNC	36	152	188	19.8	13.8–25.5
Controls	30	46	76	40.0	28.4–51.4

3.1. Prevalence of dysbiosis

The corrected prevalence of dysbiosis among patients with HNC and healthy controls is presented in Table 1. Dysbiosis was diagnosed in 36 of 188 HNC patients (19,8%, 95% CI: 13,8%–25,5%) and in 30 of 76 healthy controls (40,0%, 95% CI: 28,4%–51,4%). The prevalence of dysbiosis was significantly higher in the control group compared to the HNC group.

3.2. Risk factor analysis

Odds ratio (OR) and their 95% confidence intervals were calculated to assess the association between dysbiosis and selected variables. The odds of dysbiosis were significantly lower in the HNC group compared to controls (OR = 0.365, 95% CI: 0.188–0.708), indicating that healthy controls were 2.8 times more likely to have dysbiosis than HNC patients.

3.3. Dysbiosis

In the group of individuals with dysbiosis, several factors were analyzed to assess potential differences between patients with HNC and the control group. A history of natural childbirth was reported in 94.4% of HNC patients (N=34) and in all individuals from the control group (100%, N=30). However, the difference was not statistically significant (two-tailed proportion test, $p = 0.188218$). Similarly, history of a breastfeeding allergy was present in 91.2% of HNC patients (N=34) and 100% of the control group (N=30), with no significant difference between the groups (two-tailed proportion test, $p = 0.096078$). The prevalence of chronic diseases was slightly higher among HNC patients (75.0%, N=36) compared to the control group (66.7%, N=30), but this difference did not reach statistical significance (two-tailed proportion test, $p = 0.458294$). A significant difference was observed in antibiotic use, which was reported by 58.3% of HNC patients (N=36) and 93.3% of the control group (N=30) (two-tailed proportion test, $p = 0.001211$). Conversely, side effects of antibiotics were more frequently reported in the HNC group (69.4%, N=36) compared

to the control group (40.0%, N=30), and this difference was statistically significant (two-tailed proportion test, p = 0.016570). Regarding medication use, 50.0% of HNC patients (N=36) and 73.3% of the control group (N=30) reported taking medications, with a trend towards significance (two-tailed proportion test, p = 0.053752). Engagement in sports activities was significantly lower among HNC patients (13.9%, N=36) compared to the control group (40.0%, N=30) (two-tailed test, p = 0.015771). No significant differences were found regarding dietary habits, reported by 27.8% of HNC patients (N=36) and 20.0% of the control group (N=30) (two-tailed proportion test, p = 0.461646). Finally, traveling was reported by 11.1% of HNC patients (N=36), while no individuals from the control group reported traveling (N=30), with a trend towards statistical significance (two-tailed proportion test, p = 0.059739).

These findings suggest that while some lifestyle and medical factors such as antibiotic use and physical activity differ significantly among individuals with dysbiosis between HNC patients and the control group, other variables, including birth history, chronic diseases, and dietary habits, do not show statistically significant differences.

4. Discussion

We presented the Dysbiosis Questionnaire, the proprietary tool developed in our department after consulting a panel of interdisciplinary specialists in 2018. The questions contained therein are not asked routinely, and not all HNC patients consider the reporting of abdominal discomfort to be important.

Dysfunction of the GM, concerning both the amount and composition of the microbiota, implies a variety of clinical symptoms. In-depth information on intestinal disorders and their contributing factors is rarely collected in the routine medical history of patients with HNC. There are established scales for intestinal ailments assessment like IBS-QOL, a specific quality-of-life measure for irritable bowel syndrome (IBS) [16] or the Dutch population Nutrition Questionnaires plus and Food Frequency Questionnaires, [17, 18], but they are not suitable for population screening and above all, they are not dedicated to HNC cancer patients.

While the intricacies of GM and cancer are being investigated at the molecular level, these population data provide insight into the HNC patients' symptoms and signs of dysbiosis, which may inform health care providers' patient interactions.

Among HNC patients, 1/3 manifested dysbiosis, while in the control group it was almost half of the study participants. The control group was recruited from family members of the ward staff and emphasis was placed on including parents and grandparents to avoid methodological errors related to age, education, and big/small-town living. But finally, it was found that the control group was a decade older than the patients, the breastfeeding rate was 5% lower, the medication rate was 10% higher, 15% more were taking antibiotics, and fewer were traveling (11% versus 6%). Thus, the control group was older and presented more comorbidities than the examined group. It cannot be ruled out that it was not insignificant for the analysis of the variables combined with dysbiosis.

The main focus was on the variables with the highest degree of association with dysbiosis. The HNC patients showed statistically significant differences regarding dysbiosis based on age; dysbiosis was diagnosed more often in older HNC patients. Moreover, our results suggest that dysbiosis has a more frequent occurrence in allergic patients with HNC. Interestingly more patients with a typical diet did not suffer from dysbiosis which shows the potential relationship between an atypical diet and dysbiosis occurrence. In healthy controls, the variables related to the occurrence of dysbiosis were natural childbirth, breastfeeding, allergy, chronic diseases, medications, traveling, and taking antibiotics. Taking antibiotics occurred to be the strongest factor bound with dysbiosis in healthy controls and when both groups were analyzed together but were not significant in HNC. So, the common risk factors for dysbiosis for both the HNC patients and control group were allergy and chronic diseases. The risk factors for dysbiosis exclusive for HNC patients compared to the control group are older age and type of diet. On the other hand, natural childbirth, breastfeeding, medications, and traveling, were not the risk factors for dysbiosis in HNC, although they were statistically significantly associated with dysbiosis in healthy controls.

The presented Dysbiosis Questionnaire, standardizes and systematizes the in-depth medical interview, and this design of the tool is novel, simple, and informative. However, it is not a definitively validated tool. The "population" value of the questionnaire, while considering both groups together, showed that some lifestyle and medical factors, such as antibiotic use and physical activity, differ significantly among individuals with dysbiosis between HNC patients and the control group. Other variables, including birth history, chronic diseases, and dietary habits, do not show statistically significant differences. The role of antibiotic therapy in HNC and diet in the control group has not been confirmed, which is probably a false negative finding and therefore would require confirmation on a larger number of people.

5. Limitations and strengths of the study

5.1. Limitations

Several methodological constraints should be acknowledged when interpreting our findings. First, the disparity in sample size between the HNC group (n=188) and the control group (n=76) may have influenced the statistical power of our analyses. Second, recruitment of control participants from family members of ward staff potentially introduced selection bias, despite efforts to match for age and demographic factors. Our data revealed

that the control group was older with more comorbidities than the HNC group, which may have confounded the relationship between certain variables and dysbiosis.

The Dysbiosis Questionnaire, while developed through interdisciplinary consultation, has not undergone comprehensive validation against established clinical standards. The binary categorization of dysbiosis (present/absent) likely oversimplifies a condition that exists on a spectrum of severity. Additionally, the absence of standardized objective biomarkers to confirm questionnaire-based findings represents a significant limitation.

As a single-center study conducted over a three-month period (IX-XI 2021), our results may not be generalizable to diverse geographic regions or clinical settings. Some potentially important associations, particularly regarding antibiotic therapy in HNC patients and dietary factors in the control group, yielded inconsistent results that require verification in larger cohorts. Furthermore, the cross-sectional design precludes drawing conclusions about causality or the longitudinal impact of dysbiosis on clinical outcomes in HNC patients.

5.2. Strengths

The Dysbiosis Questionnaire itself represents a novel clinical tool developed through interdisciplinary collaboration, specifically designed for HNC patients. This standardized assessment tool systematizes the collection of dysbiosis-related data that is typically overlooked in routine clinical encounters. By covering diverse variables including birth history, dietary patterns, medication use, and chronic conditions, the questionnaire captures the multifactorial nature of dysbiosis in a clinically practical format.

Our analytical approach identifying distinct risk factor profiles between HNC patients and controls represents an important contribution to personalized patient care. The identification of age and dietary patterns as HNC-specific risk factors while establishing allergy and chronic disease as common risk factors across populations provides valuable clinical insights.

Furthermore, this work addresses an important knowledge gap at the intersection of otolaryngology and gastroenterology, potentially improving holistic care for HNC patients by drawing attention to previously unrecognized comorbidities that may impact treatment outcomes and quality of life.

6. Conclusion

A mosaic of factors turns out to have an impact on the link with dysbiosis in HNC patients. The proposed Dysbiosis Questionnaire identifies these factors accurately and may be helpful in a quick selection of prone individuals (initial diagnosis).

Authors' contribution

Research concept and design: PN, MW, JJ
Supervising the project: MW, JJ
Carrying out the experiments: -
Acquisition of data: PN, NZ
Data analysis and interpretation: PN, MW, NZ, JJ
Writing - Original Draft Preparation: PN, NZ
Writing - Review and Editing: MW, JJ
Visualization: -
Literature review: PN, NZ
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Conflict of interest

The authors have no potential conflicts of interest to declare.

Ethics approval

The protocols involved concerning the patients in this study were approved by the Ethics Committee of the Poznan Medical University, (decision number 870/20). All subjects were voluntarily recruited and informed of the nature of the study before enrollment. Written informed consent was obtained from all study subjects.

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