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ORIGINAL RESEARCH

Is There Interplay between Human Gut Microbiota and Upper Airway Susceptibility to Recurrent Respiratory Papillomatosis Exacerbations?

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Abstract

Recurrent respiratory papillomatosis (RRP) is a chronic disease caused by HPV 6 and 11 viruses, but there is still no consensus on the factors that may influence its course.

The aim is to analyze recurrent RRP activity measured by the number of surgeries and recent exacerbations of the disease, compared with the history of intestinal symptoms.

The study included 73 patients with confirmed papillomatosis. The main baseline variables were: Age of onset, breastfeeding in infancy, number of surgeries throughout life, diet, number of antibiotic treatments in the last year, BMI and current disease activity on the three-point Dikkers scale. Variables important for the analysis of the impact of intestinal dysbiosis on the course of papilloma were: Intestinal symptoms and blood test results. The primary outcome measure in this study was the association between papilloma activity and indicators of gut microbiota status.

Our results showed that diet had a statistically significant effect on disease activity measured by the interval to the last recurrence of RRP (p = 0.005). The remaining variables had no effect on the number of surgeries performed during the patient's lifetime and the current RRP status. The acquired knowledge may contribute to further work on discovering the relationship between the state of intestinal microflora and the course of RRP infection in patients with recurrent respiratory papillomatosis.

Keywords

HPV, Microbiota, Recurrent respiratory papillomatosis, RRP

Introduction

Recurrent respiratory papillomatosis (RRP) has a long-term natural history with exacerbations and remissions of varying duration. Low-risk human papillomaviruses (HPV) 6,11 are proven to be responsible for the development of RRP and other papillomata in oro-genital and respiratory mucosa, [1,2] as well as genetic autosomal recessive, a syndromic form of Juvenile RRP associated with an *NLRP1* GOF mutation [3]. However, there is still no answer to the question whether there is a factor that promotes the activity of the HPV virus and intensifies the course of the disease. The viral load in particular anatomical sites results from a combination of acquisition and clearance [4,5]. Unfortunately, a reliable correlation of protective immunity or immunologic frailty to HPV does not currently exist. In recent years, there has been compelling evidence that failure of HPV clearance and severity of RRP disease is associated with an impaired immune response. Failure to eliminate HPV 6 and 11 due to a defective immune responsiveness was proposed to play a major role in the development of RRP; dominance of cytotoxic T

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cells, activated NK cells, high numbers of stressed MIC A/B expressing lymphocytes, an overall suppression of cytokine mRNA production in RRP patients, compared to healthy controls was proved [6-11]. Growing evidence indicates that the gut microbiota participates in the maturation of the immune system and plays a key role in host defense against pathogens [12]. Thus, the intestines can be defined as the "abdominal brain" and its effects on distant organs and broadly understood homeostasis consist of: the central nervous system (brain-gut axis), control of neurotransmitters, psychosomatic diseases, neurodegenerative diseases, hormonal balance, immunity, incidence of infections.

The problem of immunological balance between HPV persistence in the respiratory tract, host immunity and intestinal microbiota has never been investigated. However, these two seemingly unrelated medical problems are closely related to the immune system. The authors' interest in the point of contact and borderline between both scientific issues is based on clinical observations. Namely, it was found that intestinal symptoms coincide with the severity of papilloma and, on the contrary, remission of the disease during the use of probiotics for reasons unrelated to papilloma. This probiotic "side effect" directed our attention to the essence of both problems, the common denominator: immunity. Therefore, the clinical symptoms of intestinal dysbiosis in RRP patients, which are indirectly associated with immune disorders, were investigated, and remissions and exacerbations were investigated during the long life of RRP. The aim is to compare RRP activity measured as the number of surgeries in real time and recent disease activity, as well as an interview regarding intestinal complaints, indicators of the state of the intestinal microbiota, and the effects of antibiotic treatments.

Material and Methods

The study was conducted prospectively at the Poznań University of Medical Science - Department of Otolaryngology and Head and Neck Surgery, the Tertiary Referral Center in the years March 2000 - February 2020.

There were 94 consecutive adult patients (41 women, 53 men) with RRP treated; out of them, 73 (39 men, 34 women) came for regular check-ups over the last 5 years and were ultimately included in the study group. A complete medical history and physical examination were conducted, taking into account RRP activity: age of onset, number of surgeries, current extent of RRP. Questions were asked about intestinal dysbiosis in terms of abdominal symptoms, irritable bowel syndrome, diarrhea, constipation, the number of antibiotic treatments in the last year and the current nature of digestive complaints with the dominant symptom. Patients underwent blood tests (classified as normal/abnormal), histological examination of a tissue sample and HPV genotyping. Data collected from the RRP patients group are presented in Table 1. The mean age of patients was 51.7 years (15-88 years). Most of the patients (69%) were diagnosed with RRP between the age of 12 and 60. Only 15% were diagnosed before the age of 12 and 16% after age 60.

The variables shown in the Table 1 were categorized as follows: Breastfed in infancy (yes, no), diet (normal, vegetarian), BMI (normal, abnormal), number of antibiotic curations in the past year (0, 1-2, > 2, N = 6), RRP disease details: Age of onset, number of surgeries in lifetime, current activity of disease in the three-point Dikkers scale [13].

In the examined group, the RRP disease severity was as follows: the age of onset was on average 37.5 (median 34), minimum 2, maximum 85, number of previously performed operations was on average 5 (median 3), minimum 1, maximum a 50; 63 patients underwent 1 to 10 operations (86%), 8 - more than 10 operations (11%) and 2 - more than 30 operations (3%). Stages 1, 2, and 3 Dikkers scale had 52 (71%), 17 (23%) respectively and 4

Table 1: Data collected from RRP study patients.

Variable	No. from cohort	% from cohort
Breastfed in Infancy		
Yes	27	36.99%
No	46	63.01%
BMI		
18.5-24.99 (normal)	31	42.47%
> 25 (abnormal)	42	57.53%
Diet		
Normal	66	90.41%
Vegetarian	7	9.59%
Intestinal Complaints		
Yes	19	26.03%
No	54	73.97%
No. of antibiotic curations/ past year	41	56.16%
0	26	35.62%
1-2	6	8.22%
> 2		
Age of onset		
< 12	11	15%
12-60	50	69%
> 60	12	16%
Surgeries/Lifetime		
< 10	63	86.30%
10-30	8	10.96%
> 30	2	2.74%
Dikkers Scale		
1	52	71.23%
2	17	23.29%
3	4	5.48%

(6%) of 73 patients had a severe course of the disease.

All procedures performed in the study are part of standard treatment in patients with RRP and are in accordance with the ethical standards of Poznan University of Medical Science and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was approved by the Bioethical Committee of Poznan University of Medical Science. Number 1010/ 18, Date 11/10/2018. Written consent, on the form accepted by the Poznań Medical Science University Ethical Committee, was taken from each patient.

Variables of fundamental importance for the RRP disease severity were: the age of onset, number of surgeries, current activity of disease.

The variables of importance for the analysis of the effect of intestinal dysbiosis on the course of RRP were as follows: intestinal complaints, number of antibiotic curations in the past year.

The main outcome measure in this study was the dependence of RRP activity (age of onset, number of lifetime surgeries and activity of disease using the Dikkers scale) and indicators of intestinal microbiota status. The project focused on interdependence between: 1. RRP activity and gut complaints, 2. RRP activity and antibiotic curations. Statistical analysis was performed using Statistica v.13.0. The Chi-square test was used for categorical data. Student's t-test and Kruskal-Wallis test and descriptive statistics were used for continuous data. The level of significance was set at $P < 0.05$.

Results

In the examined group of 73 RRP patients 54 (74%) patients did not have and 19 (26%) had intestinal complaints. Predominantly abdominal pain (9), diarrhea (4), alternating diarrhea (4), flatulence (6) were revealed. Over the past year, patients had undergone antibiotic curations: once, 40 patients (54.8%); twice, 21 patients (28.8%); three times, 6 patients (8.2%); and 8 patients (8.2%) underwent four curations. The BMI in 42 patients was normal (58%) and in 31 (42%) was abnormal (26.04 on average, median 25.54). 66 patients had a normal diet, 7 patients had a diet categorized as vegetarian.

Table 2: The interdependence between RRP disease severity and indicators of intestinal microbiota status.

Group of patients (cohort) N = 73	**Age of onset (mean)**	**p-value**	**Surgeries/ Lifetime (mean)**	**p-value**	**Interval to last RRP recurrence (mean)**	**p-value**	**RRP advancement Dikkers scale 1, 2 or 3 p-value**
Intestinal complaints Yes N = 19	40.05	0.545053	3.48	0.184190	37.11	0.134346	0.96129
No N = 54	36.57		6.17		22.00		
No. of antibiotic curations/past year							
no N = 40	41.7	0.33410	4.55	0.07990	19.63	0.46920	0.37954
1-2 N = 27	33.19		5.15		34.15		
> 2 N=6	28.67		13.0		31.0		
BMI							
Normal							
N = 30	39.3	0.546556	4.5	0.366249	16.13	0.063371	0.27317
Abnormal							
N = 43	36.2		6.14		32.77		
Diet:							
Normal	36.18	0.111380	5.50	0.906504	21.98	0.005250	0.63078
N = 66							
Normal							
N = 7	49.71		5.17		63.14		
Breastfed							
Yes N = 27	34.85	0.424316	4.44	0.380891	20.67	0.364632	0.63042
No N = 46	39.02		6.07		29.02		

Additional data to more thoroughly describe the examined group was obtained. In all 73 patients, blood tests were categorized as normal (level of IgA and IgG). In all tissue samples taken, RRP was confirmed in histology. HPV genotyping showed the predominance of HPV type 6 in 58 and HPV type 11 in 15 patients.

The basic question we wanted to answer is whether the presence of intestinal complaints and implicitly indicators of microbiota status had an impact on the course of RRP in the patients' lifetime, measured as: onset of disease, number of surgeries, interval to last RRP recurrence and recent extent of disease measured in a three-level Dikkers scale. 14 (26.92%) patients with gut compliments and 38 without (73.08%) had the first level, 4 (23.53%) and 13 (76.47%) had the second level respectively and 1 (25.0%) and 3 (75.0%) had the third level respectively. There was no statistically significant difference between gut compliments and the extent of RRP p = 0.96129. Considering the two groups of patients: With and without intestinal complaints, they didn't differ as to RRP onset, number of lifetime surgeries, nor mean interval to last RRP recurrence. Details are shown in Table 2.

Another question we wanted to answer was whether the number of antibiotic treatments correlated with the course of RRP in the patient's life. Considering three groups of patients with different frequencies of antibiotic intake in the last year (1, > 2, < 2), there was no correlation with RRP current extent of RRP (Table 2).

The final question we wanted to answer was whether current BMI values correlate with the course of RRP measured by the number of operations performed thus far and the extent of RRP. Considering the two groups of patients: With and without a normal BMI, no correlation was found. Considering BMI with a gender correction and BMI with an age correction there was no impact on the number of operations performed in the patient's lifetime and current extent of RRP.

Considering intestinal ailments with a gender correction and intestinal ailments with an age correction, there was no impact on the number of operations performed in the patient's lifetime and current RRP status.

The extent of RRP in the interval to last RRP recurrence was considered depending on breastfed history, diet and patient BMI. Considering the extent of RRP, There was no statistically significant difference between breastfed patients and the extent of RRP (p = 0.630) and There was no statistically significant difference between BMI and the interval to last RRP recurrence p = 0.063371, however the extent of RRP in the interval to last RRP according to diet, in 7 patients with vegetarian diet, the period without RRP recurrence was longer (63.14 months) than in patients (66) with normal diet (21.98 months). Diet had a statistically significant impact on recent activity of disease measured by the interval to last RRP recurrence (p = 0.005).

Discussion

The hypothesis about the common denominator of RRP activity and gut dysbiosis was based on clinical observations. The authors noticed a convergence of intestinal ailments and papilloma exacerbations and remission of the disease during the use of probiotics for RRP unrelated reasons. This "side effect" of the use of probiotics directed attention to the potential coincidence of both problems.

Premises for undertaking this research are the unsolved immunodeficiency background of HPV clearance/persistence phenomenon, unexplained impact of gut microbiota on local and systemic immunity of HPV infection, possible common denominator of HPV-related diseases escalation and gut microbiota alterations, related to lifestyle and civilization problems. Visconti, et al. estimated that the microbiome was involved in a dialogue between 71% of fecal, and 15% of blood, metabolites [14,15]. The question arose of how RRP and intestinal dysbiosis are associated, dependent, or based on gut microbiota. That is why we have undertaken clinical observations of intestinal dysbiosis in a group of patients with RRP.

To our best knowledge, this is the first research devoted to the existence of the potential upper airway-gut axis and connection between RRP activity and gut-induced immunity. Two variables were considered important for the analysis of potential intestinal microbiota status in the course of RRP. The first variable gathered widely described intestinal complaints. The second variable was the number of antibiotic curations in the past year, which should negatively affect the host microbiota. We have not noticed that intestinal complaints were most common in patients with severe RRP infection. But patients with intestinal complaints noted that the complaints usually preceded the onset of an RRP relapse, unfortunately, we could not prove it statistically. In this research, the authors proved that the vegetarian diet had a statistically significant impact on recent activity of disease measured by the interval to last RRP recurrence and patients who were vegetarian had a significantly milder course of the disease.

The interplay between the human gut microbiome and host metabolism [14-16], cross-talk with microorganisms, including bacteria, viruses, protozoa [17], influence on respiratory health, alterations in HIV infection [18] and the influence for impairment of immunity to antiviral vaccines [19] were found. But none of these studies concerned the effect of a microbiota on the course of HPV infection. The new knowledge generated by this project concerns providing an approximation to treatment resistant RRP etiology by adding another element of knowledge - clinical

coexistence of HPV related digestive complaints and their association.

To summarize, based on clinical observations, a relationship between intestinal complaints and intensity of RRP relapse was noticed. Parallel to intestinal complaints, the increase in RRP symptoms was observed. In this research, the authors did not succeed to get the statistically significant correlations between RRP activity (age of the onset, number of lifetime surgeries and current RRP activity) and the indicators of intestinal microbiota status, probably due to the limited group size. But very importantly, hard evidence concerning diet was obtained. We proved that the vegetarian diet had a statistically significant impact on lower recent activity of disease.

Our research was limited by its clinical character, based only on observation of a group of patients and not supported by correlations of RRP status with immunological tests of patients' blood and stool microbiota analysis. Possibly, there are unknown mechanisms governing the microbiota-mediated impact on immune efficacy of upper airway mucosa clearance. However, our clinical observation is a documented premise for continuing this direction of research.

The strength of research is an absolutely innovative subject, a large and carefully documented research group. We found promising clinical observations, which indicate further directions of research. The immunological potential of the mucous membrane for clearance/implantation of the HPV virus in RRP patients, gut dysbiosis and probiotic implementation should be further studied.

Conclusions

A relationship between intestinal complaints and intensity of RRP relapses was noticed. The acquired knowledge may contribute to further work on the discovery of the relationship between the state of the intestinal microbiota and the course of RRP infection in patients with recurrent respiratory papillomatosis.

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Declarations

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Conflict of interest

None declared.

Ethical approval

All procedures performed in the study are part of standard treatment in patients with RRP and are in accordance with the ethical standards of Poznan University of Medical Science and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was approved by the Bioethical Committee of Poznan University of Medical Science. Number 1010/18, Date 11/10/2018.

Author Contributions (names must be given as initials)

HK and JJ wrote the main manuscript text; MW have made substantial contributions to the conception and design of the work; NZ prepared tables; HK, JJ, NZ have made contributions in the acquisition, analysis, or interpretation of data.

All authors reviewed the manuscript.

References

1. Omland T, Akre H, Vårdal M, Brøndbo K (2012) Epidemiological aspects of recurrent respiratory papillomatosis: A population-based study. Laryngoscope 122: 1595-1599.
2. Buchinsky FJ, Donfack J, Derkay CS, Choi SS, Conley SF, et al. (2008) Age of child, more than HPV type, is associated with clinical course in recurrent respiratory papillomatosis. PLoS One 3: e2263.
3. Drutman SB, Haerynck F, Zhong FL, Hum D, Hernandez NJ, et al. (2019) Homozygous *NLRP1* gain-of-function mutation in siblings with a syndromic form of recurrent respiratory papillomatosis. Proc Natl Acad Sci USA 116: 19055-19063.
4. Beachler DC, Sugar EA, Margolick JB, Weber KM, Strickler HD, et al. (2015) Risk factors for acquisition and clearance of oral human papillomavirus infection among HIV-infected and HIV-uninfected adults. Am J Epidemiol 181: 40-53.
5. Beachler DC, Waterboer T, Pierce Campbell ChM, Ingles DJ, Kuhs KA, et al. (2016) HPV16 E6 seropositivity among cancer-free men with oral, anal or genital HPV16 infection. Papillomavirus Res 2: 141-144.
6. Holm A, Nagaeva O, Nagaev I, Loizou C, Laurell G, et al. (2017) Lymphocyte profile and cytokine mRNA expression in peripheral blood mononuclear cells of patients with recurrent respiratory papillomatosis suggest dysregulated cytokine mRNA response and impaired cytotoxic capacity. Immun Inflamm Dis 5: 541-550.
7. Wu X, Wang G, Chen X, Zhang J, Zhao J, et al. (2016) Impaired T cell-dependent humoral immune response associated with juvenile-onset recurrent respiratory papillomatosis progression. Sci Rep 6: 36378.
8. Ivancic R, Iqbal H, deSilva B, Pan Q, Matrka L (2020) Immunological tolerance of low-risk HPV in recurrent respiratory papillomatosis. Clin Exp Immunol 199: 131-142.
9. Ivancic R, Iqbal H, deSilva B, Pan Q, Matrka L (2018) Current and future management of recurrent respiratory papillomatosis. Laryngoscope Investig Otolaryngol 3: 22-34.
10. Allen CT, Lee S, Norberg SM, Kovalovsky D, Ye H, et al. (2019) Safety and clinical activity of PD-L1 blockade

in patients with aggressive recurrent respiratory papillomatosis. J Immunother Cancer 7: 119.

11. Tjon Pian Gi RE, Ilmarinen T, van den Heuvel ER, Aaltonen LM, Andersen J, et al. (2013) Safety of intralesional cidofovir in patients with recurrent respiratory papillomatosis: an international retrospective study on 635 RRP patients. Eur Arch Otorhinolaryngol 270: 1679-1687.

12. Heng Y, Kim MJ, Yang HJ, Kang S, Park S (2019) Lactobacillus intestinalis efficiently produces equol from daidzein and chungkookjang, short-term fermented soybeans. Arch Microbiol 201: 1009-1017.

13. Dikkers FG (2006) Treatment of recurrent respiratory papillomatosis with microsurgery in combination with intralesional cidofovir--a prospective study. Eur Arch Otorhinolaryngol 263: 440-443.

14. Visconti A, Le Roy CI, Rosa F, Rossi N, Martin TC, et al. (2019) Interplay between the human gut microbiome and host metabolism. Nat Commun 10: 4505.

15. Willman M, Kobasa D, Kindrachuk J (2019) A Comparative Analysis of Factors Influencing Two Outbreaks of Middle Eastern Respiratory Syndrome (MERS) in Saudi Arabia and South Korea. Viruses 11: 1119.

16. Amsterdam D, Ostrov BE (2018) The Impact of the microbiome on immunosenescence. Immunol Invest 47: 801-811.

17. Wypych TP, Wickramasinghe LC, Marsland BJ (2019) The influence of the microbiome on respiratory health. Nat Immunol 20: 1279-1290.

18. Crakes KR, Jiang G (2019) Gut microbiome alterations during HIV/SIV infection: Implications for HIV Cure. Front Microbiol 10: 1104.

19. Hagan T, Cortese M, Rouphael N, Boudreau C, Linde C, et al. (2019) Antibiotics-Driven Gut Microbiome Perturbation Alters Immunity to Vaccines in Humans. Cell 178: 1313-1328.