European Archives of Oto-Rhino-Laryngology (2019) 276:801–803
https://doi.org/10.1007/s00405-019-05351-6

LARYNGOLOGY

Experimental research on the therapeutic effect of MMR vaccine to juvenile-onset recurrent respiratory papillomatosis

Yu Wang[1] · Pei-Dong Dai[2] · Tian-Yu Zhang[3]

Received: 7 January 2019 / Accepted: 17 February 2019 / Published online: 26 February 2019

Abstract

Objective To evaluate the efficacy of MMR vaccine in the treatment of juvenile-onset recurrent respiratory papillomatosis as adjuvant therapy by experimental research.

Methods Thirty-one children with RRP were enrolled and assigned randomly to intervention group or control group. Fifteen subjects in intervention group were treated with local application MMR vaccine on the lesion after surgery; sixteen subjects in the control group were treated with surgical excision alone. The quantity of virus of positive specimens was measured by fluorescence quantitative polymerase chain reaction.

Results After treatment with MMR vaccine, viral load of intervention group was $(9.56 \pm 11.03) \times 10^8$ copies/ml, that of control group was $(22.01 \pm 17.78) \times 10^8$ copies/ml, and there was significant difference between the two groups ($P = 0.040$).

Conclusions Local application MMR vaccine as adjuvant therapy can reduce HPV viral load significantly. It is suggested that the MMR vaccine may inhibit replication of HPV DNA, but the curative effect needs further confirmation.

Keywords MMR vaccine · Recurrent respiratory papillomatosis · Experimental research

Introduction

The treatment of JORRP (Juvenile Onset Recurrent Respiratory Papillomatosis) can be summarized as surgery and adjuvant therapy. The primary treatment of JORRP is surgical excision using microlaryngoscopy, removal with powered microdebrider [1–3]. Stern's study [4] suggests that the cellular immune response may be compromised in children with JORRP and the immune status may be associated with number of papillomas sites and frequency of recurrence. Immunological adjuvant therapy may open new perspectives for innovative treatment. MMR Vaccine (Measles Mumps Rubella Vaccine) is a vaccine against measles, mumps and rubella. The literatures reported that the vaccine had significant effect in the treatment of RRP [5–7], it suggested that vaccine might enhance immune function to a certain extent.

Besides, in the clinical research [8] using the randomized controlled methods, the MMR vaccine showed some benefit in the treatment of juvenile-onset recurrent respiratory papillomatosis as adjuvant therapy by comparison of operative interval, final staging score, and number of lesions. This experimental research is to further investigate the efficacy of MMR vaccine in the treatment of juvenile-onset recurrent respiratory papillomatosis.

Major's study [9] suggests that viral load of human papillomavirus (HPV) is relatively high in cases of recurrent respiratory papillomatosis (RRP).Viral load may be related to virus replication. However, there are differences in viral load among different RRP patients. It may reflect differences in antiviral immunity. Donne et al. [10] find that variance in HPV copy number could be important to prognosis. Based on these reasons, viral load is used as an indicator for treatment and prognosis. To further explore the effectiveness of MMR vaccine for JORRP, this experiment will detect the viral load of all specimens and carry on the comparative analysis between the two groups.

✉ Yu Wang
dissugar@outlook.com

[1] Department of Otolaryngology-Head and Neck Surgery, The First Affiliated Hospital of Kunming Medical University, 295 Xichang Road, Kunming 665000, China

[2] Research Center, Eye and ENT Hospital of Fudan University, 83 Fenyang Road, Shanghai 200031, China

[3] Department of Otorhinolaryngology, Eye and ENT Hospital of Fudan University, 83 Fenyang Road, Shanghai 200031, China

Materials and methods

Thirty-one children with RRP were enrolled and assigned randomly to intervention group or control group. Fifteen subjects in intervention group were treated with local application MMR vaccine on the lesion after surgery; sixteen subjects in the control group were treated with surgical excision alone. Twenty-five fresh specimens of JORRP were collected and placed in sterilized bottle, and then stored at − 80°. Among them, there were 14 cases in intervention group and 11 cases in the control group.

The small size of papilloma tissue was placed into the 1.5 ml centrifuge tube, and then added with solution I and 20 µl protein kinase of DNA extraction kit and oscillated evenly, so that the tissue suspended in the solution, and finally cracked at 65° overnight. After cracking, the centrifuge tube was placed in the centrifuge and transiently centrifuged, added with solution II and mixed, and then centrifuged for 5 min at 14,000 rpm after setting aside for 2 min. The supernatant was removed and mixed fully with 60 µl solution III, static for 10 min, and centrifuged for 1 min at 14,000 rpm, and absorbed 1 µl supernatant as PCR amplification template. According to the proportion below for each person, 17.5 µl PCR mixture, 0.5 µl DNA polymerase and 2 µl template were mixed, and each reaction set negative control and positive control. The sample DNA, negative control, positive control and quantitative standard were put into the PCR reaction tubes and centrifuged transiently. The PCR reaction tubes were transferred to fluorescent quantitative PCR, and recorded the order of samples. All the samples were subjected to 45 cycles of amplification (denaturalization for 15 s at 95°, annealing for 50 s at 58°, extension for 5 s at 38°), and the instrument displayed automatically the results.

Results

Among the 31 patients enrolled, tissue of 6 patients was few and no fresh samples were collected, so 25 specimens of papilloma were collected. While in the middle of 25 specimens collected, specimens of 3 patients in the experimental group were collected only before the treatment. When comparing the viral load difference of 2 groups, the remaining 22 cases were analyzed after excluding 3 cases. To exclude the influence of HPV typing, age and severity of disease before treatment, we analyzed whether there were differences between the two groups in these factors. As shown in Table 1, there were four cases of HPV type 6 and seven cases of HPV type 11 in the experimental group. The age of the patients was (4.18 ± 4.22) years, and score was (15.82 ± 6.72) points before treatment. In the control group, two cases were HPV type 6, and nine cases were HPV 11 type. The age of the patients was (6.09 ± 4.18) years, and the score was (17.91 ± 8.75) points before treatment. The results showed that there was no significant difference between the two groups in the HPV typing, age and severity of the disease before treatment. Then the viral load of the 22 patients was statistically analyzed. As shown in Table 2, the viral load of the experimental group ranged from 2.75×10^8–3.42×10^9 copies/ml, with an average of $(9.56 \pm 11.03) \times 10^8$ copies/ml; the viral load of the control group was 3.75×10^8–6.39×10^9 copies/ml, with an average of $(22.01 \pm 17.78) \times 10^8$ copies/ml. The P value was 0.040 ($P < 0.05$), indicating that there were differences in viral load between the two groups after treatment, and the viral load of the experimental group was lower than that of the control group.

Table 1 Comparison of HPV typing, age and severity before treatment in two groups

	Intervention group (cases)	Control group (cases)	P value
HPV typing (HPV6/11)	4/7	2/9	0.635
Age (years)	4.18 ± 4.22	6.09 ± 4.18	0.299
Scores before treatment	15.82 ± 6.72	17.91 ± 8.75	0.537

Table 2 Comparison of viral load in two groups

Groups	Number of cases	Viral load (10^8 copies/ml)
Intervention group	11	9.56 ± 11.03
Control group	11	22.01 ± 17.78

$P = 0.040$

Discussion

The clinical symptoms are associated with the severity of the disease, and the more severe the disease is, the more likely the patient will undergo more surgeries. The purpose of treatment is to extend the interval of recurrence and reduce the severity of the disease as much as possible. Therefore, the recurrence time and the score are often used as indicators to evaluate the curative effect. The frequency of recurrence and the severity of the disease are closely related to the activity of the virus. Clinical trials [8] in the early stage of this study showed that the MMR vaccine had a certain

effect on prolonging the recurrence time and reducing the score, but there was no statistical difference between the two groups. To further explore the efficacy of MMR vaccine in patients with JORRP, the viral load of the two groups after treatment was compared to evaluate the replication of the virus. Previous literature [11–15] on JORRP treatment, regardless of which adjuvant treatment is, mostly by local injection, subcutaneous injection and oral or intravenous administration. In addition, the experimental study with control is rare, so the observation indicators are the time of relapse and the change of score after treatment. Local application can avoid complications, but the absorption and persistence of drugs have some limitations, and the difference between the two groups is difficult to show obvious clinical efficacy. After eliminating the influence of HPV typing, the viral load of two groups was detected to observe the effect of the drug from a micro-perspective. The results showed that two group had no significant difference in HPV typing, age and severity of the disease. In addition, the random clinical experiment can avoid the interference of human factors to some extent. Therefore, the data between the different groups are comparable and can be statistically analyzed. Samples of 22 patients were detected by fluorescence quantitative PCR. The results showed that the viral load of the experimental group was 2.75×10^8–3.42×10^9 copies/ml, and the average was $(9.56\pm11.03)\times10^8$ copies/ml, that of the control group was 3.75×10^8–6.39×10^9 copies/ml, and the average viral load was $(22.01\pm17.78)\times10^8$ copies/ml. The viral load in the two groups was statistically different ($P=0.040<0.05$). Thus, the viral load in the experimental group was lower than that of the control group, indicating that the MMR vaccine might inhibit the replication of HPV. Although the mechanism of MMR vaccine in the treatment of JORRP is unclear, it reflects that the MMR vaccine can alleviate JORRP to some extent.

Conclusion

Compared with surgical excision alone, local application MMR vaccine as adjuvant therapy shows some benefit in treating recurrent respiratory papillomatosis to a certain degree. It can reduce HPV viral load significantly. It is suggested that the MMR vaccine may inhibit replication of HPV DNA, but the curative effect needs further confirmation.

Compliance with ethical standards

Ethical approval This study was approved by the Ethics Committee of EYE & ENT Hospital of Fudan University. Before entering the clinical trial, parents of these JORRP patients signed informed consent (see appendix for details at the end of the article) and volunteered to join the research. Therefore, the data and materials related to this study are available. There is no funding for this study, and there is no conflict of interest between researchers and between each researcher and institution. All the authors have no competing interests to declare. The study was registered in the Chinese Clinical Trial Registry. Details are as follows: Registration number: ChiCTR-TRC-11001370. Date of Registration: May 27, 2011 (Retrospective registration).

References

1. Pasquale K, Wiatrak B, Woolley A et al (2003) Microdebrider versus CO_2 laser removal of recurrent respiratory papillomas: a prospective analysis. Laryngoscope 113(1):139–143
2. El-Bitar MA, Zalzal GH (2002) Powered instrumentation in the treatment of recurrent respiratory papillomatosis: an alternative to the carbon dioxide laser. Arch Otolaryngol Head Neck Surg 128(4):425–428
3. Patel N, Rowe M, Tunkel D (2003) Treatment of recurrent respiratory papillomatosis in children with the microdebrider. Ann Otol Rhinol Laryngol 112(1):7–10
4. Stern Y, Felipovich A, Cotton RT et al (2007) Immunocompetency in children with recurrent respiratory papillomatosis: prospective study. Ann Otol Rhinol Laryngol 116(3):169–171
5. Pashley NR (2002) Can mumps vaccine induce remission in recurrent respiratory papilloma?. Arch Otolaryngol Head Neck Surg 128(7):783–786
6. Ling Gao H, Cao M, Dong et al (2006) Clinical observation of three drugs in the treatment of laryngeal papilloma. J Clin Otorhinolaryngol 20(6):273–274
7. Ling Gao M, Dong X, Cheng (2006) Analysis of 59 cases: surgical excision plus measles vaccine injection in the treatment of laryngeal papilloma. Shandong Med J 46(30):56–57
8. Yu Wang P, Dai T, Zhang et al (2006) Clinical research on the therapeutic effect of MMR vaccine to juvenile-onset recurrent respiratory papillomatosis. Chin J Clin Oncol 33(24):1397–1400
9. Major T, Szarka K, Sziklai I et al (2005) The characteristics of human papillomavirus DNA in head and neck cancers and papillomas. J Clin Pathol 58(1):51–55
10. Donne AJ, Hampson L, Homer JJ et al (2010) The role of HPV type in recurrent respiratory papillomatosis. Int J Pediatr Otorhinolaryngol 74(1):7–14
11. Healy GB, Gelber RD, Trowbridge AL et al (1998) Treatment of recurrent respiratory papillomatosis with human leukocyte interferon. Results of a multicenter randomized clinical trail. N Engl J Med 319:104–107
12. Leventhal BG, Kashima HK, Weck PW et al (1988) Randomized surgical adjuvant trial of interferon alfa-n1 in recurrent papillomatosis. Arch Otolaryngol Head Neck Surg 114:1163–1169
13. McGlennen RC, Adams GL, Lewis CM et al (1993) Pilot trial of ribavirin for the treatment of laryngeal papillomatosis. Head Neck 15:504–513
14. Kashima H, Leventhal B, Mounts P et al (1988) Interferon alfa-n1 (wellferon)® in juvenile onset recurrent respiratory papillomatosis: results of a randomized study in twelve collaborative institutions. Laryngoscope 98(3):7
15. Pransky SM, Albright JT, Magit AE (2003) Long-term follow-up of pediatric recurrent respiratory papillomatosis managed with intralesional cidofovir. Laryngoscope 113:1583–1587