Clinical Significance of Low 2-Methoxyestradiol Levels in Serum and Tissue of Recurrent Juvenile-Onset Laryngeal Papillomatosis

Danling Liu, MD¹, Jiadong Wang, MD¹, and Yanan Xu, MD, PhD¹

Abstract

Objective. We aim to explore the correlation between serum and tissue 2-methoxyestradiol (2-ME-2) levels and recurrence of juvenile-onset respiratory papillomatosis (JORRP).

Study Design. Retrospective cohort studies.

Settings. Laboratory of Otolaryngology, Department of Head and Neck Surgery, Renji Hospital, School of Medicine, Shanghai Jiaotong University.

Subjects and Methods. Sixty-four patients diagnosed with JORRP in our department from January 2007 to December 2012 were enrolled. Patients were divided into recurrence and nonrecurrence groups, with 32 patients in each group. ELISA detected the concentration of 2-ME-2 in serum and tissue samples collected during the first surgical procedure. Mann-Whitney analysis, receiver operating characteristic curves, logistic regression model, and Kaplan-Meier method were used for data processing.

Results. There was no difference in the serum 2-ME-2 concentration between the groups (P = .237), while the tissue 2-ME-2 concentration of the recurrent group was significantly lower than that of the nonrecurrence group (P = .0001). When the area under the curve was 0.752, the cutoff value of tissue 2-ME-2 at 670.02 pg/mL yielded the highest predictive sensitivity (71.9%) and specificity (71.9%). Regrouped by this cutoff point, patients with a lower tissue 2-ME-2 level (n = 26) had shorter disease-free survival and a higher recurrence odds ratio than patients with a higher tissue 2-ME-2 level (n = 38; P = .0408, odds ratio = 7.667).

Conclusion. A low tissue 2-ME-2 level is associated with a higher recurrence rate of JORRP. Tissue 2-ME-2 may be an effective target for JORRP treatment and a convenient measure for recurrence monitoring.

Keywords

juvenile-onset recurrent respiratory papillomatosis, 2-methoxyestradiol, disease-free survival time, recurrence
changes. Active downstream estrogenic metabolites produced by estradiol 4- or 16α-hydroxylase can activate estrogen receptors, stimulating the growth of hormone-dependent tumors in target organs, which is positively correlated with tumor latency. However, the study of estrogenic metabolism for the pathogenesis of recurrence in JORRP has rarely been reported.

2-Methoxyestradiol (2-ME-2) is a naturally occurring terminal product of 17β-estradiol through A-ring metabolism. 2-ME-2 has been identified as a novel potential antitumor agent in recent reports because of its multiple antitumor activities and little affinity with estrogen receptors. Several studies have suggested a strong dosage-dependent antiproliferation and antiangiogenesis effect during in vitro and in vivo tests on multiple malignant tumors. Because different tumors express diverse sensitivities to 2-ME-2 and a dosage-dependent effect of 2-ME-2 has yet to be determined, we conducted the current study to evaluate the relationship between recurrence of JORRP and serum and tissue 2-ME-2 concentrations and then assess the clinical significance of 2-ME-2 in JORRP.

**Material and Methods**

**Clinical Information**

Sixty-four pediatric patients who were diagnosed with JORRP and underwent surgical treatment in our department (Laboratory of Otolaryngology, Department of Head and Neck Surgery, Renji Hospital, School of Medicine, Shanghai Jiaotong University) from January 2007 to December 2012 were enrolled chronologically. They were all diagnosed at first onset by our hospital. In all cases, histopathology confirmed typical laryngeal papilloma structure without any malignant transformation. All participants were either native Shanghai residents or permanent residents in Shanghai and agreed to long-term follow-ups and telephone interviews in our hospital. We excluded patients with other respiratory lesions or malignancies in the respiratory tract or other body systems, as well as patients who had received medical therapy or other adjuvant treatments within 1 year prior to surgery.

Patients enrolled by us who met the requirements were assigned to either the recurrent group or the nonrecurrence group based on whether a recurrence occurred within the 3-year follow-ups after first surgery. We enrolled 110 patients in total to achieve a desired balance between the recurrence and nonrecurrence groups and a sex balance in each group. Sixty-four pediatric patients who were diagnosed with JORRP and underwent surgical treatment in our department were enrolled. Written informed consent was obtained from each participant’s legal guardian and is available for verification through the medical records department of our hospital.

**Sample Preparation**

Blood samples from all patients were collected in the morning before breakfast and 1 day before first surgery. The samples were stored in Vacutainer tubes without EDTA for serum separation and then centrifuged at 4°C with a relative centrifugal force of 3000 × g for 15 minutes. The supernatant serum samples were transferred into clean 1.5-mL Eppendorf tubes and stored at –80°C. Tissue samples from all patients were collected immediately from remnant surgical laryngeal papilloma specimens at the end of their first surgery and stored in liquid nitrogen in <3 minutes after collection. Then, all samples were stored at –80°C. One milliliter of phosphate-buffered saline was added to 0.5 g of tissue, which was then homogenized and centrifuged at 10,000 × g for 30 minutes. The supernatant was diluted with phosphate-buffered saline and used for the assay. The homogenates were boiled for 10 minutes and centrifuged at 10,000 × g for 10 minutes to remove any interfering factors from this assay before being used for routine measurement.

**Follow-ups**

Telephone interviews were conducted, and electronic fiber laryngoscopy was used in follow-up examinations. All patients after surgery underwent laryngoscopy before discharge and again 1 month later. In 3-year follow-ups, we asked patients about their situation and informed them of the appointment time for their next laryngoscopy through telephone interviews once every 3 months. Laryngoscopy was performed every 3 months during the first follow-up year and every 6 months during the next 2 follow-up years. Whenever patients showed the symptoms of hoarseness or tone changes, they were reexamined by laryngoscope in our hospital.

In our study, patients in the nonrecurrence group underwent laryngoscopy once a year after 3-year follow-ups. Patients in recurrent group underwent multiple surgical procedures but no adjuvant therapy (Table 1).

**Table 1. Clinical Information for Recurrent and Nonrecurrence Groups.**

<table>
<thead>
<tr>
<th>Sex</th>
<th>Recurrent Group</th>
<th>Nonrecurrence Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Female</td>
<td>16</td>
<td>16</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age, mo</th>
<th>44.4 ± 17.8</th>
<th>52.1 ± 20.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intersurgical interval, mo</td>
<td>4.1 ± 1.3</td>
<td>—</td>
</tr>
<tr>
<td>Recurrences</td>
<td>2.8 ± 0.6</td>
<td>—</td>
</tr>
<tr>
<td>Tracheotomy cases</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>

a Both groups received surgical treatments. Values are presented as n or mean ± SD.
2-ME-2 Measurement

2-ME-2 levels were determined with a human 2-ME-2 EIA kit (No. 582261; Cayman Chemical Company, Ann Arbor, Michigan) according to the manufacturer’s instructions. Samples were mixed with internal standard working solution and incubated at 4°C overnight. Then, several cleanup steps were performed. Next, the samples on a 96-well plate were read at 405 nm with a microplate reader (Denley Dragon Wellsan MK 3; Thermo Scientific, Vantaa, Finland). Finally, the 2-ME-2 concentrations were calculated per the standard curve.

Statistical Analysis

Values are presented as mean ± SD unless otherwise indicated. Sample size was calculated with NCSS-PASS 15 (NCSS, Kaysville, Utah). Statistical analysis was performed mainly with SPSS 23 (IBM, New York, New York). T test for differences of clinical characters, Mann-Whitney U test for 2-ME-2 concentration, and receiver operating characteristic (ROC) curves with a binary logistic regression model for the clinical diagnostic value of 2-ME-2 were applied. Based on the tissue cutoff value, 64 patients were regrouped into low and high 2-ME-2 groups to assess disease-free survival with a Kaplan-Meier curve. Differences were considered significant when P < .05.

Results

No difference was detected in serum 2-ME-2 concentration between recurrent patients (1401.98 ± 1016.19 pg/mL) and nonrecurrence patients (1688.08 ± 1179.11 pg/mL, P = .235). However, tissue 2-ME-2 concentration showed a significant decrease among recurrent patients versus nonrecurrence patients (581.68 ± 470.85 vs 1214.78 ± 869.73 pg/mg, P < .01; Figure 1).

The ROC curve for serum and tissue 2-ME-2 levels discriminated between recurrent and nonrecurrence patients (Figure 2). The areas under the curve were 0.586 (95% CI, 0.445-0.727) and 0.752 (95% CI, 0.632-0.872), respectively. According to the highest Youden’s index, the cutoff point
for tissue samples was set at 670.02 pg/mg. It also showed the highest sensitivity (71.9%) with the highest specificity (71.9%) in predicting recurrence.

After patients were regrouped according to their tissue cutoff values, patients in the low 2-ME-2 group (n = 26) had significantly shorter disease-free survival than those in the high 2-ME-2 group (n = 38; \( P = .04 \); Figure 3).

In our regression model, the level of tissue 2-ME-2 shows a significant effect on the recurrence rate (\( P < .05 \)). The odds ratio suggests that patients with tissue 2-ME-2 < 670.02 pg/mg had 7.667-times higher odds of recurrence (95% CI = 2.524-23.284).

Discussion

JORRP carries the potential risks for airway compromise, anesthesia mishaps, and malignant transformation, as well as an emotional and economic burden on patients and their families due to the need for repeated surgical procedures, evaluations, and sometimes adjuvant treatments.1

The course of JORRP can vary from spontaneous remission to aggressive invasion with estrogenic metabolism changes.2 However, estrogen metabolism and estrogen receptor signaling are rarely evaluated in the study of JORRP recurrence. In this study, we observed a correlation between estrogenic metabolism level and recurrence of JORRP by using ELISA to capture serum and tissue concentrations of 2-ME-2—a terminal estrogenic derivative performing multiple antitumor activities—in children with JORRP. Our results show that tissue 2-ME-2 concentration is significantly lower in patients of the recurrent group than in ones of the nonrecurrence group even though the difference in serum 2-ME-2 concentration between the groups is insignificant. 2-ME-2, as a promising antitumor agent, manifests multiple functions, such as proliferation inhibition, angiogenesis, and microtubule destruction, as observed multiple times in vitro and in vivo experiments. Hypothetic mechanisms of 2-ME-2 include inhibition of HIF-1α-induced transcriptional activation of VEGF expression, microtubule disruption, angiogenetic inhibition with or without ERs, upregulation of dephospho-β-catenin in parallel with Bcl-2, and downregulation of cyclin D1. The inhibitory effect of a high concentration of 2-ME2 on cell proliferation was observed in malignant and nonmalignant cell cultures.13,15,16 This inhibitory effect can be biphasic, as reported by Lee et al and Mabjeesh et al. At a low dosage, 2-ME-2 acts as a stimulant of tumor growth, whereas at a high dosage it exhibits the reverse effect on MCF-7 human breast tumor cells.15-17 Therefore, in our future studies, we should explore further to determine the exact effect of 2-ME-2 at various concentrations. Also, how 2-ME-2 works needs to be considered carefully case by case for patients of other racial backgrounds. Several clinical studies partially confirmed the antitumor potency of 2-ME-2 if combined with other tumor-suppressing agents—for example, docetaxel in prostate cancer, recurrent and metastatic breast cancer, and other solid malignancies.13,18,19 Additionally, as illustrated by the results from our study, further explorations of 2-ME-2 and its signaling pathways in the recurrence of JORRP may illuminate the therapeutic significance of 2-ME-2 for patients with relapsed JORRP.

In the next step of our study, we attempted to use the ROC curve and Youden’s index to discover a proper 2-ME-2 concentration that may indicate the recurrence risk. Our ROC curve analysis revealed that, when the cutoff point of tissue 2-ME-2 concentration is 670.02 pg/mg, the highest sensitivity and specificity for prognosis of recurrence in JORRP were 71.9% and 71.9%, respectively. After patients were regrouped per this cutoff value, the low tissue 2-ME-2 group showed a shorter disease-free survival time than the high tissue 2-ME-2 group. The cutoff point yielded by the highest Youden’s index confirmed the effective performance of this cutoff point in our diagnostic test. This suggests the potential utility of tissue 2-ME-2 concentration as a sensitive and significant indicator for assessing prognosis and monitoring recurrence of JORRP.

In conclusion, a low tissue 2-ME-2 level has a strong association with a higher recurrence rate of JORRP. Tissue 2-ME-2 level may also have significance for evaluating clinical approaches and prognosis of JORRP.
Author Contributions
Danling Liu, ELISA, drafting the work, revised article, final approval of the version to be published; Jiadong Wang, acquisition, analysis, or interpretation of data for the work, final approval of the version to be published; Yanan Xu, design of the work, revised article, final approval of the version to be published.

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References