

The use of mitomycin-C for respiratory papillomas

Clinical, histologic and biochemical correlation

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ABSTRACT

Objective: To assess the clinical effects of mitomycin-C (MMC) on human papilloma virus (HPV)-infected tissues of the airway.

Methods: We included 10 patients with previous histologic diagnosis of recurrent respiratory papillomas (RRP) in this prospective study, conducted at the Department of Otolaryngology-Head and Neck Surgery, University of Alexandria, Egypt, between January 2000 and December 2002. Under general anesthesia, each patient underwent laser excision of all visible papillomas, followed by topical application of 1 cc of 0.5 mg/ml MMC. The procedure was repeated weekly until no visible papillomas could be microscopically detected. We histopathologically studied the obtained specimens and tested for the presence of HPV DNA using polymerase chain reaction (PCR) technique. We collected blood samples from all patients and from another 10 healthy volunteers for determination of serum interleukin-2

(IL-2) level using enzyme-linked immunosorbent assay technique.

Results: We achieved clinical remission in 8 of the patients (80%), a fact that was confirmed histopathologically and by PCR data. The mean serum IL-2 levels \pm SD was significantly lower in papilloma patients (83.6 ± 28.83 pg/ml) than in control subjects (196.3 ± 42.46 pg/ml) ($p < 0.01$). Among patients with RRP, serum IL-2 levels \pm SD was lower in initial samples (83.6 ± 28.83) than follow-up (95.7 ± 27.98 and 112.3 ± 33.8 and 129.4 ± 34.04) and remission samples (154 ± 37.84 pg/ml).

Conclusion: Our result suggests that topical application of MMC may act adjunctively to laser surgery for RRP.

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Recurrent respiratory papillomatosis (RRP) is a disease with potentially morbid consequences due to its recurrence tendency and the possibility of airway compromise. For more than 2 decades human papilloma virus (HPV) has been recognized as the etiological agents associated with the upper respiratory tract papillomas.¹ Human papilloma virus are a heterogenous group of more than 100 different viral genotypes, approximately 40 of them

are highly trophic for the epithelial cells of skin and mucous membrane; hence, they are capable of infecting human mucosal epithelia producing benign proliferations such as skin warts, anogenital condyloma, and respiratory papillomas. We can classify the HPVs into high, medium, and low risk types depending on their transforming properties. The high risk types of HPVs (16, 18 and 30) exert

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their oncogenic potentials through transforming proteins that bind to and inactivate the protein products of the tumor suppressor genes P53, thus interfering with the normal cell proliferation control mechanisms. Respiratory infection with high risk HPV apparently introduces a long-term risk of squamous cell carcinoma development, even in the absence of conventional cofactors. Low risk HPV infection (subtypes 2, 6, 11) may also act in association with these cofactors to promote carcinogenesis.^{2,3}

The postulated mechanisms by which papillomas recur include infection from distant sites, stimulation of latent virus, and incomplete removal of papillomas from the surgical site. It has been demonstrated that clinically normal mucosa surrounding papillomas can harbor latent virus, and residual viral genome remains after all gross evidence of papillomas has been vaporized.⁴

Despite multiple therapeutic protocols such as alpha interferon, cidofovir intralesional injection, indol-3-carbinol, photodynamic therapy, and pulsed dye laser, no consistently effective treatment for RRP is available.^{1,5-7}

Mitomycin-C is a naturally occurring antibiotic derived from *Streptomyces caespitosus*. It has been traditionally used as a systemic chemotherapeutic agent. Recently, it has been implicated as a topical agent in the management of condition in which fibrosis and lesion reformations are problematic. The antitumor activity of MMC is related to its ability to act as an alkylating agent inhibiting the synthesis of DNA. Topically, MMC has been shown to reduce scar formation in a variety of clinical setting.⁸ It has been most described in the ophthalmology literature, in terms of enhancement of the surgical treatment of glaucoma, dacryocystorhinostomy, and prevention of pterygia reformation after primary excision.^{9,10} Recently, the clinical use of MMC was reported in the otolaryngology literature as an adjuvant in sinus surgery, endolymphatic sac surgery, enhancing patency of myringotomy and prevention of laser-induced laryngeal fibrosis.¹¹⁻¹⁴ The exact mechanism by which MMC exerts an anti fibroblast activity is unknown. There is evidence to suggest that the reduction of fibroblast activity may be mediated by apoptosis, which is a gene-directed process causing cell death.⁸ Our report on the use of MMC for management of RRP aim to assess the ability of MMC to eradicate HPV-infected tissues from the airway, and to determine whether a correlation exists between histopathologic changes in the respiratory mucosa, the circulating levels of interleukin-2 (IL-2), the presence of HPV genome in infected tissues as detected by polymerase chain reaction (PCR) and the clinical course of RRP.

Methods. Ten patients with a previous histologic diagnosis of RRP were included in this prospective study from January 2000 to December 2002 at the Department of Otolaryngology-Head and Neck Surgery, University of Alexandria, Egypt.

All patients had recurrent lesions during the course of therapy. Adult patients who presented with single papilloma with no previous histologic diagnosis of RRP were excluded from the study. In addition, 10 normal volunteers were assigned as control subjects. The age of patients ranged from 4 - 19 years with an average of 7 years, and the duration of disease ranged from 1 - 5 years. Only one patient exhibited adult-onset disease, while the remaining 9 patients had a juvenile-onset papillomas. All patients presented with typical characteristics of RRP, and all of them required multiple surgeries to maintain a functional airway, including previous tracheotomy in 2 patients, one of them was tracheotomy dependent. The number of surgically treated recurrence (STR) per year ranged from 3 - 7 with an average of 5 STR. Eight of the 10 patients (80%) experienced aggressive disease with more than 4 STR per year. A manual form of the staging system advocated by Derkay et al¹ has been applied to assess the severity of disease and response to therapy in the studied patients. At the start of the research, all the studied patients were assigned as having bulky lesion (score 3). The total clinical score ranged from 7 - 19 with an average of 12. A total of 68 specimens and 68 blood samples were obtained from the patients until achieving clinical remission. The number of endoscopic procedures ranged from 5 - 8 for each patient with an average of 6 procedures. Eight patients achieved clinical remission that was identified by the absence of visible papillomas, improved voice quality, maintained airway, and decannulation of tracheotomy tube. Follow-up duration after remission ranged from 12 - 18 months with an average of 15 months.

Under general anesthesia, each patient underwent standard microscopic carbon dioxide (CO₂) laser excision of all visible papillomas, followed by topical application of 1 cc of 0.5 mg/ml MMC. To create a 0.5% (saturated) solution, vials containing 5 mg of MMC powder were mixed with 10 ml sterile water. A MMC-soaked pledget was applied to the injured mucosa immediately after laser induction and it was left in direct contact with the mucosa for 3 minutes. These specimens were categorized as "initial specimens." The procedure was repeated weekly until no visible papillomas could be detected microscopically, and the obtained specimens were categorized as "follow-up specimens." Two months after disappearance of all papillomas, the procedure was repeated and the tissue samples were categorized as "remission specimens".

Pathologic study. Specimens were immediately fixed in 10% buffered formalin, paraffin-embedded and processed in 4 mm sections, which were then stained with hematoxylin and eosin stain. Histopathologic examination of the specimens included a comparison of the morphologic changes between initial disease, follow-up, and remission specimens.

Polymerase-chain reaction for human papilloma virus. DNA extraction. The DNA was extracted from paraffin-embedded blocks of all specimens. The tissue was cut of the paraffin block with a scalpel, finely sliced and then deparaffinize with 1 ml xylol at room temperature for 10 minutes. After mixing and centrifugation, the xylol was removed and the tissues were washed with 1 ml of 95% ethanol alcohol and lastly air dried for 15 minutes. Specimens were incubated in DNA lysis buffer (10 mM Tris pH 8.3, 400 mM NaCl, 1% SDS, 2 mM EDTA and 20 mg/ml proteinase K) for 18 hours at 56°C before phenol, chloroform, isoamyl alcohol with a ration of 25:24:1 extraction. The extracted DNA was then precipitated with 95% ethanol, washed with 70% ethanol, air dried and then suspended in TE buffer according to the standard protocol.

General primers (GPs). GPO5/GPO6 (GPs) [5-TTTGTTACTGTGGTAGATACTAC-3], [P5-GAAAAATAAACTGTAAATCA-3] were used to screen for HPV-DNA. These primers cover GPV types 6, 11, 16, 18, 31 and 33 (the expected PCR product size is 150 bp). The positive samples were subsequently analyzed using HPV-16 E6A1B/E6A2 primers (5-GGATCCACAGGAGCGACCCAGAAG-3) and (5-CTGCACTGGGTTTCTCTACGTGTT-3) specific for HPV-16 open reading frame (the expected PCR product size is 447 bp). Polymerase chain reaction was carried out in a 50 ml reaction medium containing 10 mM Tris-HCl, 1.5 mM MgCl₂, 50 mM KCl, 0.1% Triton X-100, 10 mM of each deoxynucleotide Triphosphate, 20 pmol of each primer and 1.25 U of DNA polymerase (promega) and 10 ml of template DNA. The program was started by initial denaturation at 95°C for 5 minutes, followed by 35 cycles (Thermal cycler: Omnigen Hybaid, United Kingdom). Each cycle included a denaturation step at 95°C for 60 seconds, an annealing step at 40°C (for GPs) and an elongation step at 72°C for 90 seconds. The final extension step was carried out at 72°C for 4 minutes.

Detection of amplification products. The PCR amplified product, were detected using 2% agarose gel electrophoresis. The gel was stained with ethidium bromide (1 mg/ml) and examined using an ultraviolet light Hofer transilluminator (Hofer Scientific instruments-San Francisco, USA).

Biochemical study. Serum samples (initial, follow-up, and remission) were obtained before

anesthesia from each patient to allow in vitro determination of serum IL-2 level using enzyme-linked immunosorbent assay technique (IL-2 ELISA kit, Diaclone research, France). Blood samples were also obtained from other 10 normal volunteers and were assigned as control subjects. All samples were allowed to clot, centrifuged immediately, and the serum was separated and stored into aliquots at -70°C until analysis for IL-2 by ELISA technique in the following manner; serum samples were thawed to room temperature, samples and standards were added in duplicate into micro titer wells coated with a specific capture monoclonal antibody. A second monoclonal antibody labeled with horseradish peroxidase was added, and after an incubation period the unbound labeled antibody is washed, a chromogen (TMB) is added, incubated again and finally the reaction is stopped by adding acid, and the color developed is measured at a wave length of 450 nm in an ELISA reader (Dade Behring-Germany) and the concentration of IL-2 was obtained from a plotted standard curve.

Data analysis and statistical method. The statistical methods used included calculation of the arithmetic mean, standard deviation and student t-test, using the software program SPSS Advanced statistics package for windows version 10.

Results. Figure 1 demonstrates an initial endoscopic view of one patient with severe aggressive RRP (a), partial reduction of papillomas size and number after first sitting of laser and MMC application (b), 4 weeks follow-up endoscopic view after laser and MMC application showing marked improvement of laryngeal mucosa with only one papilloma detected at left vocal process (c), and same patient 2 months after disappearance of all papillomas (remission) showing normally healed laryngeal mucosa with no scarring or webbing (d).

Gross pathologic picture. Initial biopsies appeared as multiple, soft fleshy and grayish white fragments. On subsequent biopsies consistency tended to be more firm, with marked decline of the sample size.

Histopathologic findings. The initial biopsies showed the following morphologic features: multiple papillomatosis of the laryngeal mucosa, where papillae were covered by acanthotic stratified squamous epithelium. This was evident in all 10 cases (Figure 2a). Koilocytosis which represents the cytopathic effect of the virus was observed in the superficial keratinocytes in 3 cases (Figure 2b). In 8 cases inflammatory cellular infiltrate appeared subepithelially, 4 of them additionally showed intraepithelial neutrophilic infiltration (Figure 2c). There was associated venous congestion and hemorrhagic changes in the papillary core. In 6 out of 10 cases, the epithelial covering showed dysplastic changes that ranged from mild to

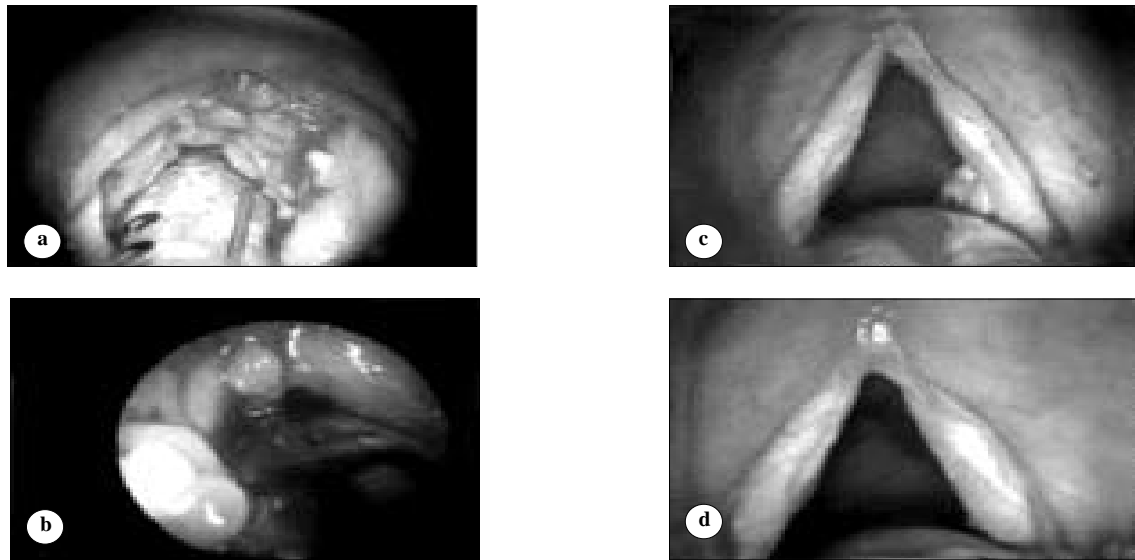


Figure 1 - Endoscopic view of a patient with recurrent respiratory papillomas (RRP). **a**) Initial view demonstrating aggressive supraglottic and glottic papillomatosis. **b**) Same patient one week after laser and mitomycin-C (MMC) application showing reduction of size and number of papillomatosis. **c**) Follow-up endoscopic view after 4 weeks MMC application demonstrating marked improvement of endolarynx with a single left vocal process papilloma. **d**) Remission endoscopic view after 6 weeks MMC application showing normal laryngeal mucosa with disappearance of all papillomatosis.

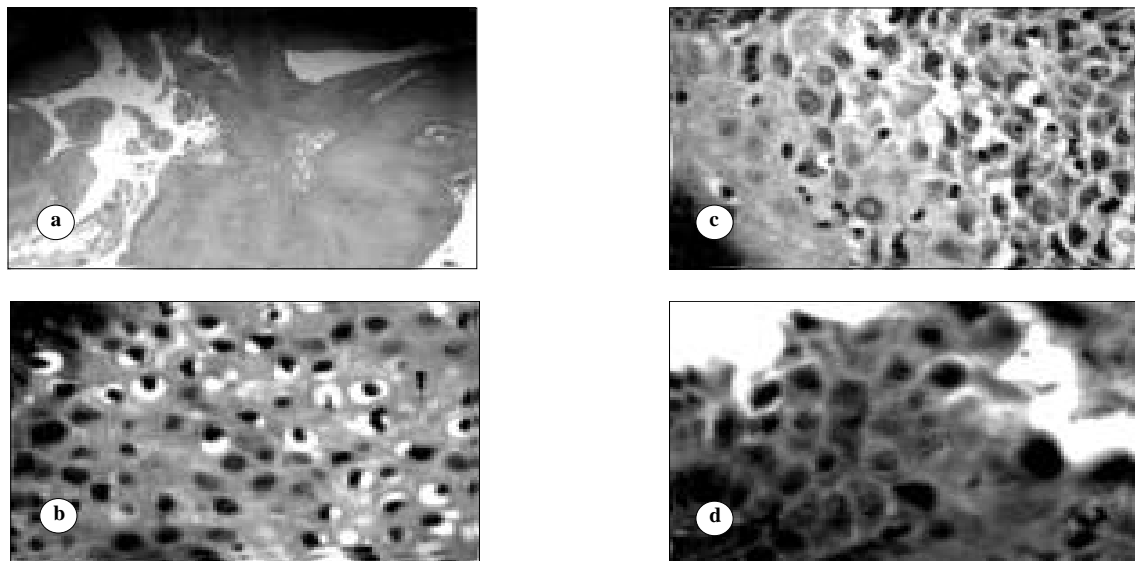


Figure 2 - Histopathologic findings in initial specimens of recurrent respiratory papillomas (RRP). **a**) Multiple papillomatosis, covered by acanthotic stratified squamous epithelium (hematoxylin and eosin stain $\times 40$). **b**) Koilocytosis, cytoplasmic vacuolization, perinuclear halo and nuclear atypia in infected epithelium (hematoxylin and eosin stain $\times 400$). **c**) Associated inflammation; intraepithelial polymorphous of the papillary covering (hematoxylin and eosin stain $\times 200$). **d**) Moderate degree dysplasia; cellular disorganization, pleomorphism, hyperchromasia and anisonucleosis (hematoxylin and eosin stain $\times 400$).

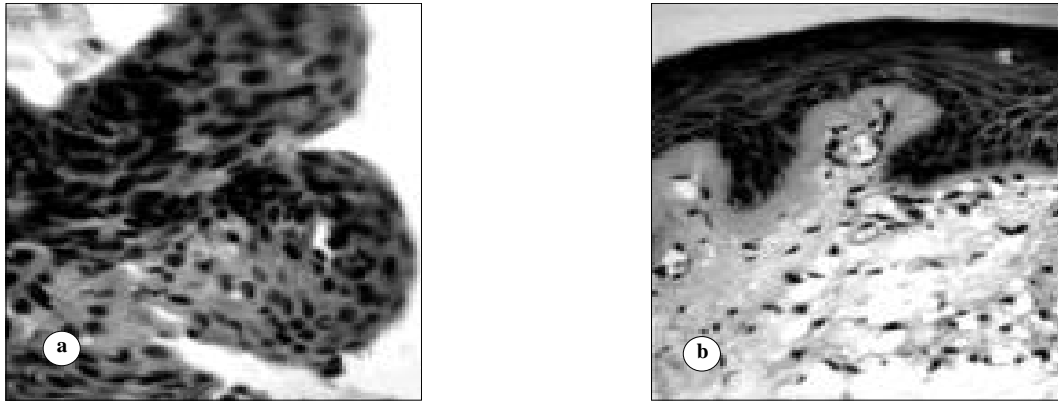


Figure 3 - Histopathologic features of remission specimens of recurrent respiratory papillomas. **a)** Small papillary formation with absence of koilocytosis as well as inflammatory changes (hematoxylin and eosin stain $\times 400$). **b)** Flattened normal laryngeal mucosa with no more papillomatosis (hematoxylin and eosin stain $\times 200$).

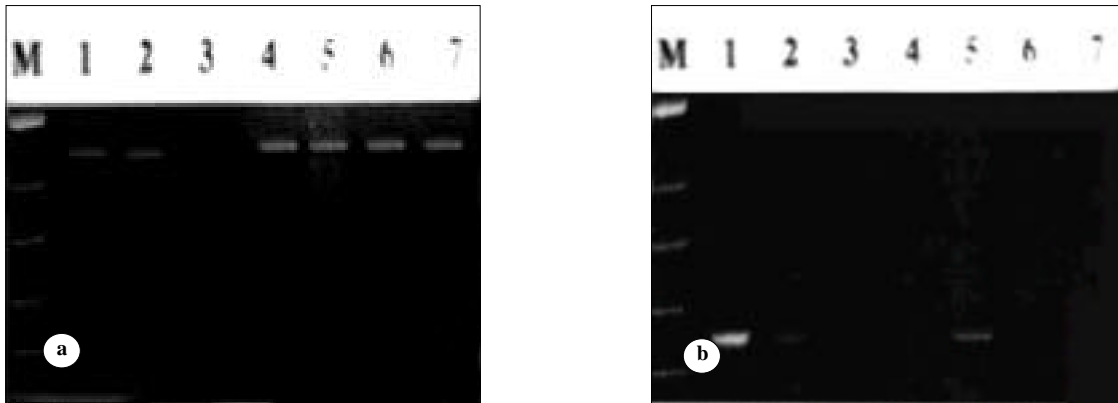


Figure 4 - Gel electrophoresis for PCR product HPV genome in initial specimens. **a)** HPV (GPs); Lanes 1, 2, and 4-6 are positive tissue extracts. Lane 3 is negative for HPV (GPs). Lane M is molecular weight marker ladder. **b)** HPV (type 16); Lane 1 showing strong result. Lanes 2 and 5 are weak results.

moderate degree dysplasia mainly of the basal and parabasal compartment (**Figure 2d**). In the subsequent follow-up specimens, there was marked decrease in the complexity of papillary formation with diminution of the number of inflammatory cells in both intra- and subepithelial compartment. The remission samples showed small solitary papilloma in only 2 cases, but with absent signs of inflammation and dysplasia (**Figure 3a**), whereas in most of the cases (8/10), normal laryngeal mucosa was evident (**Figure 3b**).

The PCR results in the initial biopsies, HPV genome was detected in 6 out of 10 cases (60%) using general primers (**Figure 4a**). All samples were negative for HPV-16 except one case who showed strong positive results, and another 2 cases that had weak results (**Figure 4b**). However, the other 3 cases were from patients with an aggressive and extensive

clinical disease. The remission samples showed HPV DNA products in only 2 cases.

Assay of serum IL-2. Serum IL-2 levels were compared in papilloma patients and normal control subjects. Differences between initial sample and the follow-up samples in papilloma patients until remissions were also noted (**Table 1**). Data analysis revealed that mean serum IL-2 levels \pm SD were significantly lower in papilloma patients than in control subjects ($p < 0.01$ significant) (**Table 2**). Among patients with PRP, serum IL-2 levels \pm SD was lower in initial sample than the follow-up samples and the remission sample. However, even after remission, IL-2 levels remained lower than those in control.

Discussion. Recurrent respiratory papillomatosis is the most common benign neoplasm of the larynx

in children. Despite its benign histology, RRP has potentially morbid consequences and is often difficult to treat due to its tendency to recur and spread throughout the respiratory tract. Despite the significant advances in the molecular-biologic characterization of the disease process, and the potential medical therapy including interferon, indol-3-carbinol and cidofovir, the mainstay of treatment of RRP has remained surgical extirpation, with laser therapy.^{1,2,15} However, even with the advantages that CO₂ laser offers, such as precise excision, bloodless field, and preserved vocal quality, long lasting clearance of the disease is difficult to achieve. One important contributing factor is the persistence of HPV DNA in areas of normally appearing mucosa that remain after laser extirpation of all visible papillomas. Glynn et al⁴ empathized that HPV DNA is consistently present following laser therapy in RRP patients. Histologic findings indicate that recurrent papilloma is likely the source of viral DNA, and it serves as a nidus for regrowth, and may explain RRP recalcitrance to conventional laser therapy. One concern in light of

such results is the possibility of contamination during laser treatment. A laser plum of RRP lesions has shown detectable HPV DNA and that clinically normal mucosa surrounding papilloma can harbor latent virus, and that microscopic papillomas remain after all gross evidence of papillomas has been vaporized. A wide variety of techniques such as immunohistochemical studies, in situ hybridization and PCR may be used for detection of viral DNA in biopsy samples. However, PCR is considered the method of choice for epidemiological investigations, and is a highly sensitive technique for determining the presence or absent of viral genome at the laser bed.¹⁶ This propensity to recur, with the risk of stenosis after aggressive laser therapy, and the possibility of inadvertently implanting HPV into any break in normal mucosa, has prompted the search for other adjunctive therapeutic modalities such as interferon, indol-3-carbinol and cidofovir intralesional injection. The rationale for selecting MMC as an adjuvant agent in the current study was based on its implication as a systemic chemotherapeutic agent for the

Table 1 - Mean and standard deviation for IL-2 levels in control and patients.

Group	IL-2 Initial samples	IL-2 Follow-up sample (1)	IL-2 Follow-up sample (2)	IL-2 Follow-up sample (3)	IL-2 Remission samples
Control					
Number	10				
Mean ± standard deviation	196.3 ± 42.4684				
Patients					
Number	10	10	10	10	10
Mean ± standard deviation	83.6 ± 28.8336	95.7 ± 27.9804	112.3 ± 33.8035	129.4 ± 34.0496	154 ± 37.8476
Total					
Number	20	10	10	10	10
Mean ± standard deviation	139.95 ± 67.7538	95.7 ± 27.9804	112.3 ± 33.8035	129.4 ± 34.0496	154 ± 37.8476

Table 2 - T-test for IL-2 levels between group 1(control) and group 2 (patients).

Variable	Levene's Test for equality of variances		T-test for equality of means						
	F	Sig	t	df	Sig (2-tailed)	Mean difference	Std error difference	95% confidence interval of difference	
								Lower	Upper
IL2_0									
Equal variances assumed	2.651	0.121	6.943	18	0	112.7	16.2325	78.2609	146.8032
Equal variances not assumed			6.943	15.843	0	112.7	16.2325	78.2609	146.1391
IL2_0 - initial IL-2 sample, F - ratio of mean squares, Sig - Significance, df - degree of freedom, Std - standard. Patients IL2_0 = 83.6 ± 28.8336 pg/ml, controls IL-2 = 196.3 ± 42.4684 pg/ml, t=6.943 and p<0.01(significant).									

management of disseminated adenocarcinomas and interstitial carcinoma. The MMC selectively inhibits DNA synthesis through its ability to cross-link DNA, much like the alkylating agents. At higher concentration, it also suppresses cellular RNA and protein synthesis.¹⁷ Topically, it has been shown to inhibit fibroblast proliferation; hence, its clinical use as a modulator of the wound healing procedure.¹⁸ Another rationale has evolved incidentally during a previous project conducted by our team investigating the inhibiting effect of MMC on laser-induced laryngeal fibrosis, where it was documented that topical application of MMC has unexpectedly augmented the laser effect on papillomas.¹⁹

In the current study, laryngeal mucosa of initial disease showed histological evidence of viral inclusion (koilocytosis), epithelial hyperplasia, in addition to associated inflammation in 8/10 cases, as well as atypia in 6/10 cases. Such histological findings were supported by PCR results that demonstrated HPV DNA genome in approximately 60% of the specimens using GPs. However, further specification of the viral subtype showed strong positivity for HPV-subtype 16 in one case, and weak positive results in another 2 cases. Gomez et al¹⁶ has proved that the presence of HPV in juvenile onset recurrent respiratory papillomas [in 50 (100%) of the cases], most frequently of subtype 6-11 group, and PCR was used successfully in archived tissue especially the highly sensitive method of nested-PCR. In a study by Gale et al²⁰ that was carried out on a series of 79 laryngeal papillomas from 36 patients; they found no clear-cut histological differences between juvenile and adult RRP. The presence of koilocytosis was equally observed in both types and there was no prevalent type of epithelial hyperplasia in either form. In the latter study, although there were a number of patients presenting atypical hyperplasia that was considered as precancerous lesion, during a 14-year follow up no carcinomatous transformation was observed.²⁰ In the studied patients, there was a marked improvement in histologic features of follow-up and remission samples. Such histopathologic findings were associated with laryngoscopic evidence of decline in the degree of papillomatosis up to disappearance of all visible papillomas in remission biopsies. The PCR documented this change as well (decreased detection of HPV DNA from 60% in initial biopsies to 20% of the remission samples); a finding that correlates with the biochemical evidences of improved immunologic state of such patients with increasing level of serum IL-2 in treated patient compared to the infected papilloma patient.

The human body primary defense against viral infection such as HPV, is the cell-mediated arm of the immune system mediated by T helper 1 (Th1) cells. The viral antigen interacts with Th1 to stimulate the T cells to produce IL2 and other

cytokines. Interleukin-2 is the primary interleukin responsible for activation of the cell-mediated (Th1) arm of immune response and is considered as the critical messenger in this cascade and initiates a specific lymphoproliferative response.²¹ A growing number of investigators propose that altered immune response may be the key to explaining the clinical behavior of HPV infection. Epidemiological data reveal an increased incidence of HPV infection in conditions in which cell-mediated immunity is suppressed. Our findings support the presence of an aberrant cell-mediated immune response in children with RRP, and are in accordance with other studies involving anogenital HPV infection. Our data support the concept that malfunction of the appropriate cell mediated (Th1) response may allow pathologic disease to develop clinically in RRP. This defect may affect a pathologic shift from Th1 to Th2.

To assess the clinical results of MMC, the current study adopted a nomenclature suggested by Dedo et al²² in 1982 describing "remission" as: failure to detect any visible papillomas by direct microscopy for 2 months or more after the surgical extirpation. Our data revealed clinical remission in 8 of the patients (80%), a fact that was confirmed histopathologically and by PCR data that failed to identify any HPV DNA in all remission specimens. Only 2 weak positive cases were detected by PCR amplification. Dedo and Yu¹⁵ in a recent study on the effect of laser on respiratory papillomatosis in 244 patients achieved "remission" (no visible RP on indirect or often direct laryngoscopy 2 months after laser removal) in 37% of patients. The higher remission rate in the current study comparative to only 37% documented by Dedo and Yu¹⁵ in their publication dealing with the use of CO₂ laser for RRP might be attributed to the ability of MMC to work as alkylating agent to inhibit protein synthesis by cross-linking. The detected data propose that MMC exerts dual action on RRP; first is antineoplastic activity being incorporated into the genome DNA viruses. Programmed cell death occurs in epithelial cells infected by replicating papillomas viruses that incorporate MMC into the viral genome; and second is anti-proliferative activity to prevent post laser granulation and scarring. Even with the tremendous advancement in the field of endolaryngeal laser surgery such as the development of the microspot manipulator allowing microprecision, hemostatic ability, and the introduction of mucosa-preserving laser surgery such as micro-trapdoor flap technique, there is further injury to the airway mucosa that leads to fibroblast proliferation and collagen depositions, which are critical for the formation of scar.^{1,15,23} A review of the literature reveals that there is a consensus for conservative laser surgery therapy aiming at palliation and maintaining a safe airway, and not at eradication of the papillomas.^{1,20,22} In our

study, however, frequent laser excisions of papillomas were not associated with excessive scarring and granulations formation. Apart from minor atrophy of vocal fold mucosa, we demonstrate a significant decrease in subepithelial granulation, as well as a notable prevention of postoperative granuloma formation, with no formation of glottic web comparative to 25% incidence reported in many series.^{15,23} In our opinion, topical application of MMC to the surgical field has augmented the laser effect on papillomas and allowed us to minimize the laser power, yet, still achieving eradication of papillomas, with minimal scarring if ever. Although 8 of our patients achieved normal voice postoperatively, however, this minimal scarring and atrophy of the vocal fold have been reported by Garrett et al¹⁷ to produce an adverse effect on the normal vocal fold vibratory pattern. They proposed that topical application of MMC not only modulated the wound healing process, but also affected the normal fibroblast products within the lamina propria, leading to this negative functional impact.

Unlike the wide range of potentially severe side effects seen with the systemic administration of MMC, our patients did not experience local or systemic side effects, this finding is in accordance with the data of several previous publications that have revealed largely mild local reactions without significant systemic toxicity.^{8,17,18} The major toxicity from MMC given systemically has been myelosuppression that can be life threatening. In the ophthalmology literature, although minor local complications have been documented, no systemic complications have been reported with topical use. In the current study, no local adverse tissue reactions were noted, and no systemic toxicities impairing patient's general health were detected.

In conclusion, our study submits the technique of topical application of MMC as a possible adjunctive to current laser surgery of RRP. Although no local or systemic toxic effects of MMC were encountered in this experimental study, more randomized trials with prolonged follow-up period should be conducted. The current data propose that a correlation exists between clinical course of RRP and histopathologic data as well as biochemical findings. More fundamental scientific research is needed to test the theory of a pathological Th1-Th2 shift in patients with RRP. Our results demonstrate that the topical application of MMC can be beneficial in the modulation of wound healing and in decreasing scar formation in the treatment of RRP.

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