The Therapeutic Effect of Gamma Interferon in Chronic Bronchiolitis Due to Mustard Gas

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ABSTRACT

Bronchiolitis has been known as one of the pathological features of lung injuries in mustard gas exposed patients

The purpose of this research was to evaluate the efficacy of interferon gamma-1b on the lung function in mustard gas exposed patients with bronchiolitis. In multicenter research interferon gamma-1b was effective in pulmonary fibrosis with unknown reason, but assessment of effect of interferon gamma-1b in the chemical injured patients has not so far been reported.

Thirty six patients with bronchiolitis whose lung lesion had been diagnosed through the chest high resolution computerized tomography (HRCT) and pathological study were divided into two eighteen member case and control groups. The case group was treated for 6 months with a combination of 200 μg of interferon gamma-1b (given three times per week subcutaneously) and 7.5mg of prednisolone (given once a day), while the control group received their previous medications (prednisolone 7.5mg/day + salbutamol and beclomethasone spray PRN).

In the two groups, FEV1 did not have statistical differences at base line (49.3±2.9 and 48.7±4.1, respectively, p = 0.6), whereas after treatment the data for FEV1 showed a significant increase in the case group (66.3±5.4) when compared with those in the control group (57.3±8.6, p=0.001).

The findings of this study indicate that a 6-month treatment with interferon gamma -1b plus a low-dose of prednisolone is associated with the improvement of the lung function in mustard gas exposed patients with bronchiolitis

Keywords: Bronchiolitis Obliterans; Interferon Gamma 1b; Mustard Gas

INTRODUCTION

Thousands of Iranians were exposed to sulfur mustard (SM) agent during Iran-Iraq war (1980-1988).[1] Currently, myriads of survivors, with different degrees of exposure, are suffering from respiratory complications.[2] Chronic bronchitis, bronchiectasis and lung fibrosis have been reported as late complications of sulfur mustard exposure.[3]

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Moreover, according to a newly published study, bronchiolitis should be considered as another long-term sequel following mustard gas exposure.[4]

Transforming growth factor-beta 1 (TGF-ß1) is known as an important contributor to the pathophisiological factor of lung fibrosis and bronchiolitis.[5]

Moreover, it has been demonstrated that the level of TGF –β1 is above normal range in bronchiolitis and lung fibrosis.[6] On the other hand, IFN-γ can also negatively regulate the transcription of selected TGF beta genes.[7]

Available evidence suggests that patients with moderate clinical manifestations may experience a shift from Th1 to Th2 cytokine patterns since

leukocyte cultures from these patients show a decrease in IFN-γ levels.[8] It has been shown previously that IFN-γ inhibits the transcription of collagen in fibroblasts independent of Statl–promoter interactions and abrogates its stimulation induced by TGF β1.[9-13] Thus, TGF- β1 and IFN-γ exert opposite effects on collagen synthesis. Since these two cytokines are secreted by inflammatory cells at the sites of tissue injury, their antagonistic interactions regulating collagen synthesis are likely to be of great importance in the maintenance of connective tissue homeostasis.

Therefore, in the light of previous observations, we assumed that 6 months of treatment with interferon gamma-1b could improve the lung function in mustard gas exposed patients, whose bronchiolitis is one of the pathological features of their lungs.

MATERIALS AND METHODS

Thirty six male patients (mean age: 38±2 years) with bronchiolitis oblitrans were tested in a randomized clinical trial study, during March 2001 to September 2002 in Baghiatallah hospital of Tehran, Iran.

All of these subjects were exposed to sulfur mustard (SM) from 1985 to 1987 during Iran-Iraq war. The documentation of SM exposure was based on official certification issued by the Veterans' (Janbazan) Foundation. None of these patients was smoker or addict.

patients with the following characteristics were included in our study: pathological findings compatible with bronchiolitis in trans bronchial lung biopsy (TBLB) resolution computerized tomography (HRCT), which was the presence of air trapping equal or more than 25% of the cross-sectional area of an affected lung on at least one scan level and at least 3 months free of immunosuppressive drug use. Patients with depression, uncontrolled vomiting, diabetes mellitus, uncontrolled hypertension and multiple sclerosis were excluded.

Having obtained additional written consents from the patients, we performed fiber optic bronchoscopy and obtained specimens of the lung during peripheral transbronchial biopsy for the assessment of lung pathology.

The effectiveness of therapeutic effects of this regime was assessed during drug administration. Therapeutic response criteria included: dyspnea indices (defined in table 1), hospitalization (the admission days in hospital over a 6 months period), and pulmonary function test (PFT) and arterial blood gas (ABG) changes at rest.[14]

Table 1. Dyspnea Index (SEPAR).

0. No dyspnea exept for very intense efforts
1. Dyspnea with accelerated on walking or when climbing a hill
2. The patient walks/ed more slowly than people of his age
3. The patient has/had to stop after walking for 5 minutes
4. Dyspnea at dressing or undressing; could not leave home

The patients were randomly divided to two groups (computer generated randomization list). The case group received 200 μg of interferon γ–1b (Imukin, Boehringer Ingelheim. Vienna, Austria) subcutaneously three times per week and 7.5mg of oral prednisolone daily for 6 months. The control group was treated with their previous drug regimen, which contained prednisolone 7.5 mg/day. Salbutamol and Beclomethasone spray were administered for them only when their symptoms exacerbated. Assessment of drug side effect was done by the doctor who examined the patients. Also patients were informed if they observed any new sign and symptom they should consult their physicians.

Pulmonary Function Test

Lung function was measured at base line and after 1-3 and 6months of treatments. The PFT tests were performed using pneumotachography with a computerized analysis of Spiro gram (Auto box DL 6200, Sensormedics, Austria). The tests were performed by experienced lung- function technicians who were blinded to our study, and all the patients were familiar with the equipment and experienced in performing maneuvers while seated with a nose clip in place.

The subjects were asked to perform at least three forced expiratory maneuvers with verbal encouragement to blow maximally throughout until they felt there was no air to expel. Both patients and technicians received visual feedback from a monitor screen during the test, which was repeated until three technically satisfactory curves with reproducible contours were obtained.[15] All the indices used for analysis were derived from the same maneuver, which was the one with the largest FVC and FEV1.

Arterial Blood Gas Evaluation

Arterial-blood gases at rest were measured with a gas analyzer (model AVL 510, Radiometer, Copenhagen, Denmark)

Chest High Resolution Computerized Tomography

Chest HRCT examinations were obtained on one scanner (Hi Speed Advantage: General Electric Medical Systems). Each HRCT examination consisted of five 1.0 mm collimation images obtained during both deep inspiration and full expiration, respectively, with the patient lying in a supine position. Images were obtained at the levels of the aortic arch, midway between the aortic arch and the tracheal carina, midway between the tracheal carina and the right hemi diagram and finally, 1 cm above the right hemi diagram.

No contrast was administered. All the images were reconstructed using a high-spatial-resolution algorithm and displayed at standard (level-700, width 1500) and narrow (level 700, width 1000) lung window settings. The HRCT scans were reviewed by a radiologist and a pulmonologist. The only data available to the HRCT reviewers were the patients' age, sex and the history of exposure to sulfur mustard. The images were interpreted simultaneously, and consensus for air trapping and mosaic parenchymal attenuation were registered. The expiratory images were assessed for the presence and lobar distribution of air trapping. The criteria used to diagnose the presence of air trapping were the alteration of normal anterior and posterior lobar attenuation gradients and/or a lack of homogeneous increase in lung attenuation resulting in persistent areas of decreased attenuation. The extent of air trapping was qualified and classified using the same system as defined for hyper lucent regions on inspiratory images, considering that limited air trapping has also been reported in normal individuals. The presence of air trapping was considered indicative of bronchiolitis only if it exceeded 25% of the cross-sectional area of an affected lung on at least one scan level. Expiratory images displayed at standard and narrow window settings were directly compared to determine the differences in the conspicuously of air trapping.

The value of these measurements was assessed based on the following definitions: improvement, stabilization, and failure. Improvement was defined when FVC and Pao2 increased more than 12% and 10%, respectively and dyspnea indices declined one stage. Stabilization was defined if FVC and Pao2 increased less than 12% and 10%, respectively and dyspnea indices had no changes. And finally, failure was defined by any decrease in the baseline of FVC and Pao2 and a one - stage increase in dyspnea index.

Histomorphological and Histochemical Changes

There was variability in histochemical and histomorphological changes due to sulfur mustard. These changes induced by inflammatory cells such as neutrophils, lymphocytes, macrophages and eosinophils which were different in the base of severity. Pathological biopsy showed collagen and hyalin sedimentation. These changes suggest inflammatory lesions such as bronchiolitis and panbronchial fibrosis (Figure 1).

Statistical Analysis

Numeric data were expressed by means of values±standard deviation. SPSS software was used to calculate the differences between the case and control groups.

RESULTS

The mean age of the case and control groups were 38±5 years and 38±1 years; the means of the first chemical exposure until the time of study for the case and control groups were 14±5 years and 14±6 years, respectively. In both groups, FEV1 did not have statistical differences at base line (49.3±2.9 and 48.7±4.1, respectively, p=0.6) Nevertheless, After treatment these values increased significantly in the case group (66.3±5.4) when compared with those in the control group (57.3±8.6) (figure 2) On the other hand, there was a considerable increase in the FVC of the case group (77.7±10) when compared with that of the control group (60.6±10.9). Other parameters of response to treatment are indicated in Tables 2 and 3.

Table 4 provides a breakdown of the values of changes in the case and control groups according to the following definitions: improvement, stabilization and failure ($p<0.05$). Post treatment HRCT shows insignificant changes, reported by radiologist. Figure 3 is sample picture of chest HRCT in a chemically induced bronchioltis obliterans patient.

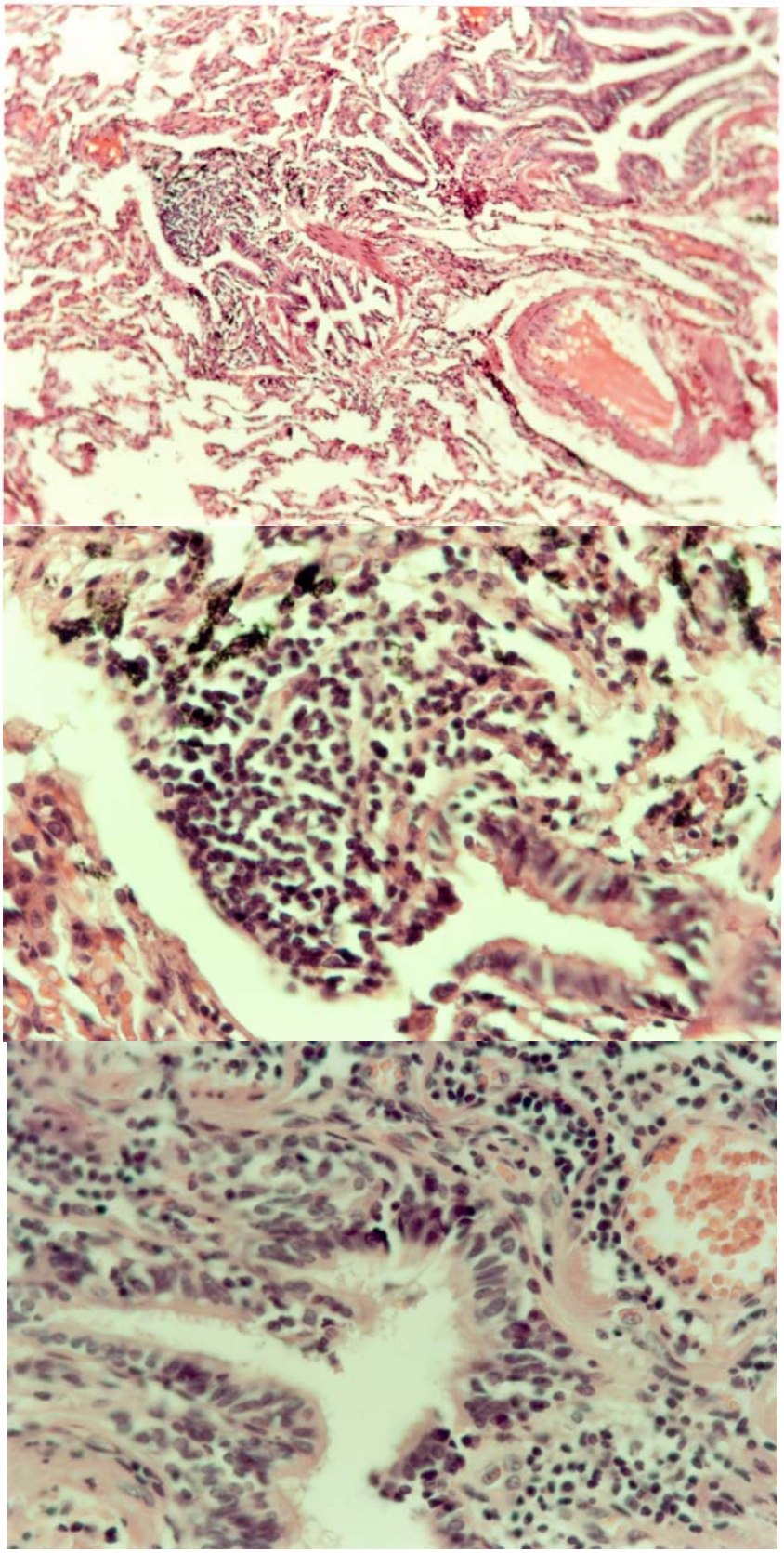

Figure 1. Histomorphological and histochemical changes due to sulfur mustard gas.

Table 2. Pre treatment and post treatment indices of Dyspnea, Hospitalization, Pao2 and FVC in case and control groups.

Indices	Case group	Control group	P-value
Dyspnea indices			
pre treatment	5.3 ± 0.4	4.9±0.7	>0.05
Post treatment	1.2±0.4	3.2±0.7	<0.05
Hospitalization			
Pre treatment	4.2±0.8	4.5±0.1	>0.05
Post treatment	1.1±0.8	4.7±1	<0.05
Pao2			
pre treatment	56.1±5	58.3±5.9	>0.05
Post treatment	65±5.2	59.5±4.3	<0.05
FVC			
pre treatment	50.9±8.2	53±8.1	>0.05
Post treatment	77.7±10	60.6±10.9	<0.05
FEV1			
pre treatment	49.3±4.3	48.7±4.2	>0.05
Post treatment	66±5.6	57.2±5.9	<0.05

Table 3. Pre treatment and post treatment indices of FEV_1 and FVC.

Patient Number	Pre FEV_1	Pre FVC	Pao2		FEV_1			FVC		
			Pre	Post	Month 1	Month 3	Month 6	Month 1	Month 3	Month 6
1	45	49	54	53	47	48	52	51	51	54
2	47	48	53	64	49	57	82	54	78	84
3	46	46	58	61	47	51	55	51	48	57
4	50	51	57	63	52	57	66	54	61	67.5
5	53	54	54	54	57	60	59	53	57	62
6	46	49	50	51	49	54	65	51	59	68
7	49	51	55	60	48	54	69	54	57.5	71
8	47	50	53	62	47	53	68	54	59.5	71
9	45	47	54	54	49	48	50	44	51	53
10	51	54	59	64	54	63	67	57	67	71
11	52	53	58	57	53	53	57	59	61	60
12	54	56	60	67	59	65	70	68	64	75
13	47	49	57	57	49	48	54	48.5	54	58
14	49	51	59	71	54	61	64	57	64	68
15	53	54	59	57	52	60	65	57	60	69
16	49	52	58	59	51	63	66	57	61	69
17	47	49	56	56	49	52	55	51	57	59
18	55	58	61	71	56	49	54	57.5	60	58.5
19	46	48	50	50	46	49	51	51	52	53
20	47	49	51	51	47	48	64	50	58	67
21	49	51	53	51	49	52.5	58	52	57	60
22	51	53	54	59	51	56	68	58	64	71
23	43	45	54	54	43	47	51	47	51	54
24	51	53	57	61	51	58	68	58	60	69
25	50	54	54	54	50	54	60	57	61	63
26	46	49	50	51	46	49	68	50	59	71
27	47	51	54	55	47	54	61	57	61	64
28	52	56	57	57	52	58	70	59	67	75
29	54	57	60	60	54	60	64	58	59	67
30	53	59	59	64	53	59	60	61	62	64
31	54	58	59	60	54	63	72	59	67	76
32	46	49	58	58	46	53	63	51	60	67
33	42	47	59	58	42	38	34	40	42	38.5
34	49	53	60	59	47	56	65	54	61	68
35	56	57	62	67	56	63	64	68	61	68
36	47	56	61	71	47	54	67	59	67	78

* The even numbers are cases and the odd numbers are controls.

Table 4. The assessment of response to treatment based on improvement, stabilization and failure definitions.

Outcomes	Case group			Control group		
	FVC	Dyspnea	Pao2	FVC	Dyspnea	Pao2
Improvement	17 (94.4%)	18 (100%)	12 (66.7%)	4 (22.3%)	6 (33.3%)	5 (27.05%)
Stabilization	1 (5.6%)	-	6 (33.3%)	13 (72.2%)	7 (38.9%)	8 (44.4%)
Failure	-	-	-	1 (5.6%)	5 (27.8%)	5 (27.5%)

DISCUSSION

To date, according to available clinical data, there has been no immunosuppressive strategy showing significant and reliable improvement of lung function in patients with BOS. On the other hand, stabilization and decline in lung function are only outcomes, following augmented immunosuppressive regimens. The results of our study supported this hypothesis that the pulmonary function tests of patients with chemically induced bronchiolitis could be improved with the administration of interferon gamma-1b. Furthermore, decrease in hospitalization time, increase in arterial oxygenation and improvement in dyspnea indices are other benefits that could be achieved with interferon gamma 1-b administration. Since according to a newly published study, TGF β1 plays a pivotal role in BO pathogenesis,[5] we assume that lung function improvement in our patients can be ascribed to the down regulating effect of interferon gamma 1-b on TGF β1.

Differentiation and proliferation of a wide variety of cells are controlled by TGF β1, which is an important component of cytokines family.[16,17] Although TGF- β1 was identified and designated on the basis of its ability to induce and transform rat fibroblasts, it is now clear that it exerts multiple effects on different types of cells.[18] Moreover, it modulates some inflammatory parameters which have a pivotal role in the genesis and maintenance of fibrotic reactions of the lung, including chemotaxis of macrophages, suppression of macrophage and lymphocyte function, chemotaxis and proliferation of fibroblasts and modulation of collagen synthesis.[19,20] In addition, TGF-β1 is a strong stimulator of extra cellular matrix synthesis. It is synthesized and released in inflamed sites by a variety of inflammatory cells (including activated macrophages, lymphocytes and platelets) which contribute to inflammatory processes of the lung.[21]

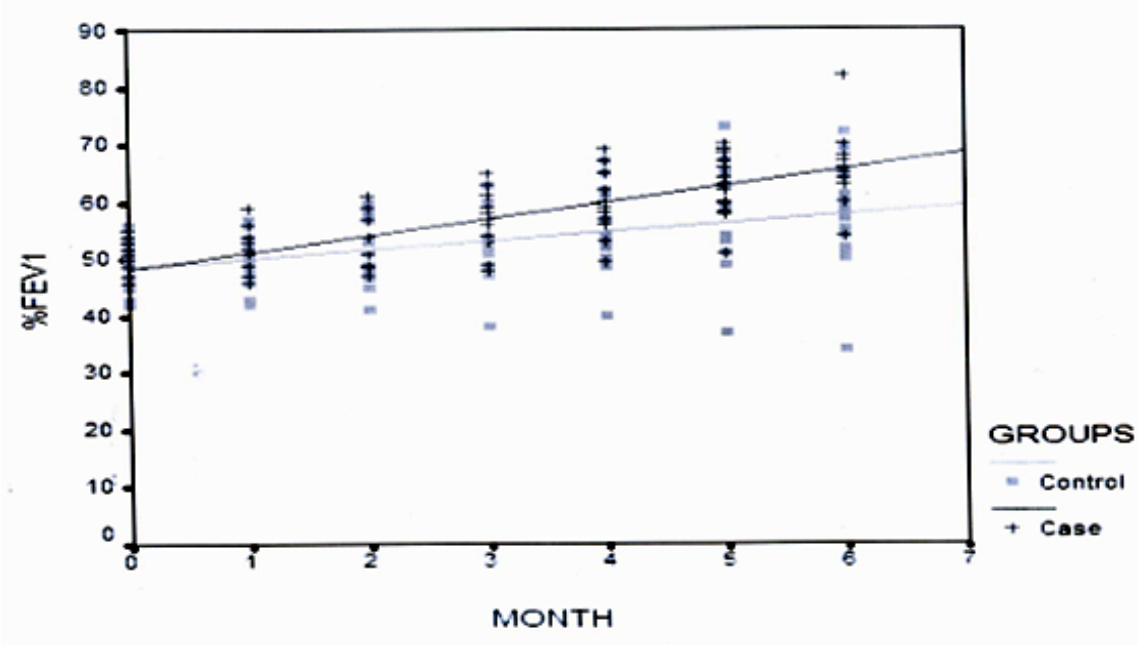

Figure 2. Distribution and regression line of %FEV1 over a 6-month period in case and control groups.

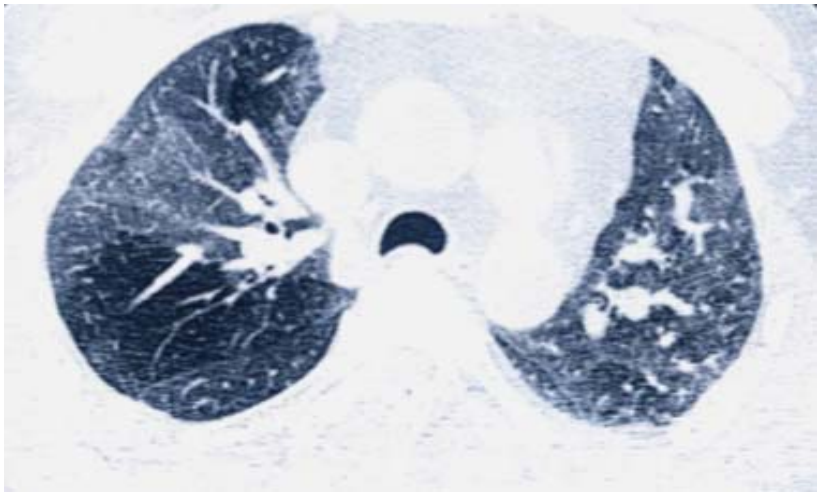

Figure 3. Air trapping in chest HRCT in expiratory phase in a mustard gas exposed case with bronchiolitis obliterans.

Several animal studies have indicated that anti TGF-β1 neutralizing antibodies and natural TGF-β1 inhibitors (e.g. decorin) can block the effects of excessive TGF-β1 activity through the inhibition of TGF- β1 binding to its receptors. Moreover, gene therapy, by inhibiting Smads proteins or dominant negative TGF-β1 receptors, and blocking TGF-β1 signaling improve the process of fibrosis in the liver and kidney.[22,23]

Another animal study, carried out on mice, has demonstrated that in Bleomycin induced pulmonary fibrosis, the expression of inhibitory Smad 7 decreases lung fibrosis.[24] In human studies, the successful treatment of diabetic nephropathy by angiotensin-converting–enzyme inhibitors, hepatic fibrosis by INF-α, autoimmune hepatitis by azathioprine and prednisone and pulmonary fibrosis by cyclosporine or interferon gamma 1-b are due in part to the ability of these drugs and cytokines to reduce TGF-β1 serum levels.[25-29] In fact, the efficacy of INF-α in treating hepatic fibrosis directly correlates with decline in serum TGF- β1 level.[30] We did not measure the TGF -β1 levels in our cases, because of the above–mentioned observations. We assumed that response to treatment in our patients can be justified with down regulating mechanisms of TGF- β1 gene expression by INF γ-1b. In multicentre research interferon gamma-1b was effective in pulmonary fibrosis with unknown reason,[29,31] but assessment of effect of interferon gamma-1b in the chemical injured patients is the first time in the world to be reported.

The results of this research open a new window for investigators studying the pathogenesis of bronchiolitis obliterans. Future immunobiological progresses will bring about satisfactory solutions to this devastating process.

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