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RESEARCH ARTICLE

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Quantification of Interleukin Level in the Workers Handling Highly Toxic Gas Phosgene

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ABSTRACT

Exposure to the toxic gases and air pollutants results in acute lung injury and inflammation in the respiratory system. Phosgene which is classified as chemical warfare agent is highly toxic and extremely reactive gas and has the potential to cause respiratory injury in the exposed population. The workers working in pharmaceutical and fertilizers industry are susceptible to its exposure and toxicity. In line with other chemical warfare agents, phosgene toxicity is predicted to be more severe with short-duration, high-concentration exposures than long-duration, low-concentration exposures.

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Introduction

Inhalation of a high dose results in neutrophil infiltration, pulmonary edema and oxidative stress in the lungs. Though the pathogenesis behind phosgene induced pulmonary edema is not clear several hypothesis such as the dysfunction of ATPase's due to impaired mitochondrial respiration [1,2], inflammatory responses in acute lung injury [3] and disruption of alveolar barrier function [4] have been suggested. The inflammatory cascade involves inflammatory cells such as Th2, mast cells, basophils, etc. and the release of inflammatory mediators. These mediators may be actively secreted from Th2, mast cells, basophils, etc. which have been recruited into the air spaces in response to the inflammatory cascade or may appear due to release from apoptosis [5,6]. These inflammatory mediators include the proinflammatory cytokines interleukins (IL) as well as the anti-inflammatory interleukins such as IL-4 and IL-6.

IL-4 is an anti-inflammatory cytokine that is important during the response against inflammatory, infectious and allergic conditions in which all of these cell types are involved [7]. The production of IL-4 is tightly regulated and restricted to T cells, mainly Th2, mast cells, basophils and activated eosinophil [8]

Interleukin-6 (IL-6) is a pleiotropic cytokine that has important role in endothelial cell dysfunction, driving chronic inflammation, autoimmunity and fibro genesis. IL-6 has a dual effect; it acts as a defense mechanism as well as a pro-inflammatory, the latter effect being mainly in chronic inflammation [9,10].

Due to stringent measures to safely handle such chemical warfare agent, the accidental acute exposure has become a rarity while working in such installations always pose a risk of chronic low level exposure. Though few reports on the acute poisoning of phosgene are available [11,12], the data on effects and estimation of inflammatory markers in human associated with chronic low-level exposure to phosgene is scarce. Thus, the present study is undertaken to assess the inflammatory markers IL-4 and IL-6 in workers exposed to chronic low level of toxic gas phosgene.

Material and Methods

The present cross-sectional study included 78 workers of a phosgene manufacturing and utilizing plant. The Institutional Ethics Committee of National Institute of Occupational Health, Ahmedabad approved the project. The information regarding demographic, occupational and clinical history was collected on a pre-designed and pre-tested preformat through interview of subject. This was followed by complete clinical examination, chest radiography and spirometry of each subject. For categorizing pulmonary

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function impairment, cut-off of 80% of predictive FVC and 70% of FEV1/FVC ratio was taken and those categorized as having restrictive and/or obstructive impairment were considered abnormal. For comparison of means, smoking habits were dichotomized as ever smoker and never smoker. Any abnormal sign on chest radiograph was considered as abnormal. The type of exposure was dichotomized into direct and indirect exposure. Direct exposure group included workers handling phosgene and other phosgene by-product while the indirect exposure group included the managerial and supervisory staff.

For interleukin estimation, 4 ml blood sample was collected in plain vacationer by venipuncture taking all aseptic precaution. The serum was separated after centrifugation at 5000 rpm for 10 minutes. The serum samples were stored at 20°C in a deep fridge till the analysis. The total human IL-6 and IL-4 were estimated using a highly sensitive Enzyme Linked Immunosorbent Assay (ELISA) kit (Ray Biotech, Inc.). 100 ml of each of standard and sample was dispensed in respective well.

The microliter wells were covered and incubated for 2.5 hours at room temperature. The content of each well was decanted and washed four times with 300 ml of washing buffer. After the last wash, any remaining Wash Buffer was removed by aspirating or decanting. The plate was inverted and blotted against clean paper towels. 100 ml of 1 x biotinylated antibody was added to each well and incubated at room temperature for 1 hour with gentle shaking. The solution from each well was discarded and washed with washing buffer. 100 m of Streptavidin solution was added to each well and incubated for 45 minutes at room temperature. The solution from each well was discarded and washes with washing buffer. 100 ml of TMB was added and incubated at room for 30 minutes in the dark. The absorbance of each well was determined at 450 nm immediately after adding stop solution using ELISA reader. A standard curve was plotted and the value of each sample was determined using the standard curve. Statistical analysis was carried out using statistical software package SPSS 24.0 and included calculation of proportion and percentages and application of tests of significance such as ANOVA.

Results

Table 1 shows the frequency distribution of IL-4 and IL-6. The normal values for IL-4 and IL-6 was 30 pg/ml and 10 mg/ml respectively. When compared with normal value only 1 (1.3%) study subject had raised IL-4 levels whereas IL-6 levels were higher than normal in 8 (10.3%) subjects. From the Table 1 it can be observed that maximum 37.2% had IL-4 levels between 15 pg/ml-20 pg/ml. Similarly, majority (64.1%) of the study subjects had IL-6 levels between 6 pg/ml-10 pg/ml.

Table 1. Frequency distribution of IL-4 and IL-6 levels in study subjects

Interleukin	Number of subject's n (%)
Interleukin-4 (pg/ml)	4(5.1)
<5.00	
5.01-10.00	9(11.5)
10.01-15.00	22(28.2)
15.01-20.00	29(37.2)
≥ 20.01	14(17.9)
Interleukin-6 (pg/ml)	1(1.3)
<4.00	
4.01-6.00	19(24.4)
6.01-8.00	25(32.1)
8.01-10.00	25(32.1)
≥ 10.01	8(10.3)

Table 2 depicts the distribution of mean IL-4 and IL-6 levels according to study parameters. The study variables included are age, duration of employment, type of exposure, chest radiography finding, Spiro metric classification and smoking habits. The overall mean IL-4 and IL-6 values were 15.65 ± 6.21 pg/ml and 7.54 ± 1.97 pg/ml respectively. It can be observed that mean values of IL-4 were non-significantly different according to study variables. However, for IL-6, those working for ≥20 years had significantly higher mean levels (F=7.34; p=0.001) of IL-6.

Discussion

The present study carried out to quantify the interleukin-4 and interleukin-6 levels in workers exposed to highly toxic gas suggested that while 8.3% workers had higher than normal levels of IL-6 only 1.3% had higher than normal levels of IL-4. It was also observed that IL-4 in one third and IL-6 in two third study subjects was toward higher border of normal

Table . Mean IL-4 and IL-6 values according to study parameter

Study parameter	n	Mean ± SD	
		IL-4 (pg/ml)	IL-6 (pg/ml)
Age (in yrs)			
<25	2	16.02	5.79
25-34	18	17.75 ± 7.26	7.78 ± 2.16

35-44	21	15.73 ± 5.5	6.83 ± 2.03
≥ 45	37	14.56 ± 6.09	7.91 ± 1.75
		F=1.07; p=0.365	F=2.03; p=0.116
Duration of employment (yrs)			
<10	16	17.13 ± 7.26	7.89 ± 2.11
Oct-19	23	16.19 ± 5.73	6.32 ± 1.51
≥ 20	39	14.71 ± 6.04	8.11 ± 1.87
		F=0.99; p=0.376	F=7.34; p=0.001
Smoking habit			
Non-smoker	58	15.66 ± 6.09	7.64 ± 1.94
Ever Smoker	20	15.59 ± 6.71	7.23 ± 2.06
	F=0.002; p=0.968	F=0.674; p=0.414	cor
Type of exposure			
Direct exposure	51	15.75 ± 5.84	7.63 ± 1.94
Indirect exposure	27	15.46 ± 6.96	7.36 ± 2.04
		F=0.037; p=0.848	F=0.345; p=0.559
Chest radiograph			
Normal	71	15.57 ± 5.75	7.59 ± 1.96
Abnormal	7	16.43 ± 10.46	6.96 ± 2.12
		F=0.12; p=0.73	F=0.66; p=0.41
Spiro metric classification			
Normal	50	16.17 ± 6.55	7.36 ± 2.07
Abnormal	28	14.71 ± 5.55	7.25 ± 1.76
		F=0.99; p=0.32	F=1.13; p=0.29
Overall Mean ± SD		15.65 ± 6.21	7.54 ± 1.97

levels. This suggest that chronic inflammation exists that can be partly attributed to low level of exposure to toxic gas. However, not so alarming levels of these biomarkers is a good sign of effectively handling such toxic gas. Very few studies reported values of inflammatory biomarkers of phosgene induced toxicity. One such study reported higher levels of IL-6 and other cytokines in phosgene exposed population [11].

The analysis of mean level of IL-4 and IL-6 according to various study variables revealed that the IL-6 level of those in the job for more than 20 years was higher than those in the job for lesser duration. This suggest that with increasing duration of job there might be chronic cumulative exposure to low dose of toxic gas stimulating the subclinical level inflammation as well as release of cytokines. The analysis of interleukin and Spiro metric measurements suggested that those having pulmonary function abnormalities had lower levels of IL-4 and IL-6. Ideally there should have been higher levels of interleukins as reported in earlier studies. IL-6 may increase IL-4 during Th2 differentiation, inducing inflammation, and it may play as a potential promoting factor for asthma as well as some other lung diseases [13,14]. The lower levels in present study can be attributed to lower number of cases with abnormalities due to lower level of exposure to toxic gas.

Though this is first study which estimated interleukin levels among those handling toxic gas like phosgene, there are some limitations in the study. The study involves a smaller sample size which was further reduced on categorization. This might have resulted in non-significant difference in mean levels according to study variables. Though the area monitoring of the workplace revealed that the phosgene exposure was within the international permissible level still the personal monitoring of toxic gas level would have given more appropriate dose response relationship.

Conclusion

Finally, to conclude this study emphasizes though acute exposure of the workers is a rarity due to effective handling of this chemical warfare, still the periodic health monitoring of such workers using biomarkers of inflammation is required to detect chronic inflammation. This is important considering the long term consequences of such exposure. Interleukins though non-specific can serve as important biomark-

ers for this inflammation especially IL-6 in chronic inflammation. A more detailed study on higher sample size is also recommended.

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Conflict of Interest

The author has no competing interests to this study.

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