International Journal of Advanced Research and Review

www.ijarr.in

THE IMPACT OF SILICA DUST EXPOSURE ON HUMORAL IMMUNITY OF EXPOSED WORKERS

Aruna A Jawade[1], Rajani G Tumane[1], Shubhangi K Pingle*[1], Bhuvneshwari A. Mehere[2]

(ajawade28@rediffmail.com, rajo_tumane@yahoo.co.in, pingle.shubhangi@gmail.com, bhumimehere@gmail.com)

National Institute of Miners' Health, JNARDDC Campus, Opposite Wadi Police Station, Amravati road, Wadi, Nagpur 440 023, (M.S.), India.[1]
Dr. Ambedkar College Nagpur, Deekshabhoomi, Nagpur, Maharashtra 440010.[2]
**Corresponding author: ***Dr. Shubhangi K. Pingle Ph.D., Assistant Director & Head of Department, Department of Biochemistry, National Institute of Miners' Health (NIMH), JNARDDC Campus, Wadi, Nagpur - 440023, INDIA. E-mail: pingle.shubhangi@gmail.com

ABSTRACT

Introduction: Several pieces of evidence focus light on the culprit characteristic of crystalline silica for the induction and development of silicosis. Therefore, the present study was suggested to assess the impact of silica exposure on immunoglobulin levels. **Material and Methods:** One hundred fifty subjects (n=150) were selected from the stone quarries of Central India, classified as healthy control (n=75) and workers exposed to respirable silica dust (n=75).Workers having less than 10 years exposure were excluded. **Result:** The mean duration of exposure for the experimental group is 15.8 ± 4.80 yrs. The mean value of serum IgM in exposed and control groups are 75.8.1l ± 18.52 mg/dL and 109 ± 19.81 mg/dL respectively and significantly depressed in the exposed group as compared to the control group ($p<0.0001$). The mean differences in the concentration of IgA between two groups are statistically significant with $P< 0.001$. Experimental group is showing 248.14±58.20 mg/dL concentration in comparison with control 124.26 ±30.11 mg/dL. The mean IgG value of exposed and control group is 1737.18 ± 469.62 mg/dL & 1005.17±219.56mg/dL respectively.

Conclusion: Concentration of immunoglobulin IgG and IgA is raised and IgM level is depleted in silica dust exposed workers. However, further studies are necessary to understand the immunological mechanisms alteration involved in silicosis.

Keywords*:* immunoglobulin, occupational diseases, silica, silicosis, stone quarries

INTRODUCTION

Dust containing crystalline silica particles generated during mining, quarrying, drilling, foundry and construction work. Long term occupational exposure to silica dust has been associated with multiple serious health issues (Chen W et al., 2012). Inhaled respirable silica particulate matters are renowned foreign bodies and give danger signal to dendritic cells (DCs) and alveolar macrophages in the lungs. It damages lung tissues; induce inflammatory response with initiation of fibrosis and scarring process. Circulating immune cells may also recurrently encounter these particles and are caused various alterations (Traboulsi H et al.,2017). Simultaneously, fibrotic repair or wound healing of lung tissue works in progress.

It enhances the deposition of extracellular matrix (ECM) components, such as hyaluronan, fibronectin, and interstitial collagens (Herrera J et al., 2018). Deposition of ECM are irreversibly disorganized lung tissue structure, thickening of the alveolar and peribronchial walls, thus leads to impairing gas exchanges in the lungs (White ES. 2012) As a result of chronic inflammation in lungs, alteration of the function of immune cells gets enhanced (Nurwidya F, et al., 2016).

Inhalation, retention and reaction of silica particles in lungs tissue progresses to Silicosis, an occupational fibrotic lung disease (Mossman BT, Churg A. 1997, Becklake MR 1992, De Matteis S et al., 2017, Nelson G, 2013, Nandi S et al., 2018) The immune cascade is known to be involved in fibrotic lung disease; it is unclear how the inflammatory process and epithelial damage ultimately contribute to chronicity and progression of pulmonary fibrosis (Marsland BJ, et al., 2011). The development of the disease is depends upon individual immunity of the subjects (Marsland AL 13 et al., 2002). Scientists reported that, there is an evidence for both enhancement and depression of immune responses along with the various symptoms of autoimmune diseases after exposure to silica (Palmer BC et al., 2019). Based on these considerations, we hypothesized that, inhalation of crystalline silica initiates a sequence of events leading not only to fibrosis, but also to dysfunction of immune system. Furthermore, we hypothesized that better characterization of immunoglobulin responses to silicosis would help to elucidate mechanisms underlying other manifestations of silicosis-associated immune dysfunction.

The rate of its progression appears to depend upon both the rate of silica deposition in the lungs and the total amount of crystalline silica actually retained in the lungs (Lee S et al., 2017). Patients with silicosis endure a significant activation of their immune system accompanied by a diminished functional immune response (Moore BB, Hogaboam CM., 2007).

Respirable Silica particle is recognized as to impair humoral and cellular immune system, at the same time it may have systemic effects in the lungs and regional lymph nodes. Higher level of circulating immune complexes, anti--nuclear antibodies and rheumatoid factor induces the production of serum polyclonal immunoglobulin that are often associated with silicosis, rheumatoid arthritis, systemic sclerosis (scleroderma) and systemic lupus erythematosus. Several lines of evidence support the view that the pathogenesis of silicosis involves uncontrolled immune processes (Nigam SK et al 1990, Watanabe S et al., 1987, Calhoun WJ 1987 et al.,1986).

Limited studies are available about the effects of silica exposure on humoral immunity. The aim of this analysis was to estimate the immune aggression in silica exposed subjects.

MATERIALS & METHODS

The study was conducted in 150 workers, in the age group between 20-60 years and exposure to silica dust was 10 to 30 years were randomly selected from stone quarries of Panchgoan, Umred, Central India. Workers were divided in two groups, healthy controls (n=75) and workers exposed to respirable silica dust (n=75). A detailed questionnaire about their life style, duration of exposure, working conditions was collected from all individual. Workers with any history of exposure to other occupations, toxic agents, and pulmonary diseases were excluded from the study. Blood samples were collected from worker and then used for further analysis.

Determination of Immunoglobulin (IgM, IgG, & IgA) by ELISA

Enzyme-Linked Immunosorbent Assay (ELISA) kit is specially designed for the quantitative measurement of immunoglobulin in plasma & serum. An immunoglobulin ((IgM, IgG, & IgA) specific antibody has been precoated onto three different 96-well plates and blocked. Standards or test samples were added to the wells and subsequently IgM, IgG, & IgA specific biotinylated detection antibodies to the respective plates were added and then followed by washing with wash buffer. Streptavidin Peroxidase Conjugate was added and unbound conjugates were washed away with wash buffer. TMB was used to visualize Streptavidin Peroxidase enzymatic reaction. TMB was catalyzed by Streptavidin Peroxidase to produce a blue color product that changes into yellow after adding acidic stop solution. The optical density (OD) of yellow colour is directly proportional to the amount of Immunoglobulins captured in plate.

Statistics

All results are expressed as mean ± standard deviation. Comparisons between silica- exposed and immunoglobulin levels were performed using student *t*-test for normally distributed data or Mann- Whitney ranksum tests for data that were not normally distributed. Statistical analysis was done by using Med-Calc software.

RESULT

Table I provides the descriptive statistics of fundamental characteristics of subjects in two study groups. As regard to age, the mean age of subjects in control group is 41.58 ± 8.63 yrs and experimental group 41.88 ± 7.75. The difference in mean age across study groups is not statistically significant by using independent sample t-test. The mean duration of exposure for subjects in experimental group is 15.8 ± 4.80 yrs. Further, the mean body mass index (BMI) of subjects across study groups differed significantly as revealed by a P-value of 0.0001 (P< 0.05) using one-way analysis of variance. The mean BMI in experimental group is 22.50 ± 4.64 kg/m2 which is significantly lower than the control (26.14 ± 5.20 kg/m2). The dietary habits of subjects showed significant association with the study groups as indicated by a P-value of 0.0001 using Chi-square test. The proportion of subjects with smoking habit in experimental group is 36 (48 %) which is significantly higher than that of control 25(19 %) group as revealed by P-value of 0.6324 (P> 0.05) using Chi-square test. In case of tobacco consumption, the proportion of subjects in control 27(36%) and experimental group is 44 (58.7%) is differed insignificantly with that of experimental group as indicated by P-value of 0.53 (P> 0.05) using Chi-square test. The proportion of subjects consuming alcohol in experimental group 49(65.3%) is higher than that of control group 27(36%) but not significant as revealed by P-value of 0.60 by using chi square test.

Serum IgM level is significantly depressed in exposed group as compared to control group (p<0.0001). The mean value of serum IgM in exposed and control group are 75.8.1l ± 18.52 mg/dL and 109 ± 19.81 mg/dL. The mean differences in concentration of IgA between two groups are statistically significant with P< 0.0001 Experimental group is showing 248.14±58.20 mg/dL concentration in comparison with control 124.26 ±30.11 mg/dL. The mean IgG value of exposed and control group is 1737.18 ± 469.62 mg/dL & 1005.17±219.56 mg/dL respectively.

The scatter plot shows positive relationship between exposure period and levels of immunoglobulins (IgG and IgA) Figure-2. Pearson's correlation coefficient for these two parameters is 0.855 and 0.933 respectively indicating statistically significant relationship between the two parameters. (P-value < 0.0001) The exposure period and IgM level shows negative relationship by Pearson's correlation coefficient (- 0.744, P-value < 0.0001).

DISCUSSION

Current remedy for the treatment of lung disease just act to slow down disease development without cure, or organ transplantation and life-long immunosuppressant treatment. Available information about the effects of exposure to silica on humoral immunity is scanty. Activation and suppression of humoral immune system to exposure of crystalline silica is very much augmentable in scientific community. (Ziskind M, et al.,1976, Zone JJ, Rom WN 1985). While it is known about the involvement of the immune response in lung fibrosis, it is unclear whether immune suppression or activation is beneficial to treatment of the disease. The theory is that exposure to silica at first will suppress humoral immune system, and chronic exposure to silica will stimulate the immunity. Karnik *et al*. was also agree with the dysfunction of humoral immune system in silicosis (Karnik AB, 1990). Scientist J. J. Zone *et. al.* reported that the asbestos workers had significantly increased levels of IgG and IgA in circulating immune complexes (S. Divya priya1 et al., 2011). Result of the present research also agrees with the above findings reported by scientists. *S. Divya Priya et al* suggested that slightly raised IgA mean level in cement factory workers was observed, these findings were reliable with the previous studies it indicate the stimulation of respiratory tracts by inhaled particulate matters and IgA played an important role in the pathogenesis of silicosis (Başaran N et al., 2002). Similar kind of result was obtained in the present study. *Başaran et.al* reported that serum IgG, IgA and IgM level was significantly lower in silica-exposed foundry workers than control group the same consistent result was found in IgM level but not agreed with levels of IgG,& IgA in the present study (silicosis (Başaran N et al., 2002).

CONCLUSION

The overall results obtained in present study are indicating the activation of humoral mediated immunity in silica exposed workers. Despite these intriguing insights, further studies are warranted to better understand the functional role of immune cell subtypes and their micro environmental and contextual interactions with epithelial cells, (myo) fibroblasts, and ECM components in the pathogenesis of silicosis.

Acknowledgements

We would like to express our gratitude to the Director of National Institute of Miners Health, Nagpur, Maharashtra, India, for granting permission to the investigators to use all infrastructure and facilities available at NIMH while conducting the research.

Conflict of interest: None

REFERENCES

Başaran N, Shubair M, Undeğer U, Canpinar H, Kars A. 2002. Alterations in immune parameters in foundry and pottery workers. Toxicology. 178(2):81-8.

Becklake MR. 1992. The mineral dust diseases. Tuber Lung Dis. 73(1):13-20.

Calhoun WJ, Christman JW, Ershler WB, Graham WG, Davis GS. 1986. Raised immunoglobulin concentrations in bronchoalveolar lavage fluid of healthy granite workers. Thorax. 41(4):266-73. PubMed

Chen W, Liu Y, Wang H, Hnizdo E, Sun Y, Su L, Zhang X, Weng S, Bochmann F,Hearl FJ, Chen J, Wu T. 2012. Long-term exposure to silica dust and risk of total and cause-specific mortality in Chinese workers: a cohort study. PLoS Med. e1001206.

De Matteis S, Heederik D, Burdorf A, Colosio C, Cullinan P, Henneberger PK, Olsson A, Raynal A, Rooijackers J, Santonen T, Sastre J, Schlünssen V, van Tongeren M, Sigsgaard T; 2017. European Respiratory Society Environment and Health Committee. Current and new challenges in occupational lung diseases. Eur Respir Rev.15;26(146).

Herrera J, Henke CA, Bitterman PB. 2018. Extracellular matrix as a driver of progressive fibrosis. J Clin Invest. 2;128(1):45-53.
Karnik AB, Saiyed HN, Nigam SK. 1990. Humoral immunologic dysfunction in silicosis. Indian J Med Res. 92:440-2.
Lee S, Hayashi H, Mastuzaki H, Kumagai-Takei N, Otsuki T. 2017. Silicosis and autoimmunity. Curr Opin Allergy Clin Immunol.17(2):78-84.
Marsland BJ, Königshoff M, Saglani S, Eickelberg O. 2011. Immune system dysregulation in chronic lung disease. Eur Respir J. 38(3):500-1.
Marsland AL, Bachen EA, Cohen S, Rabin B, Manuck SB. 2002 Stress, immune reactivity and susceptibility to infectious disease. Physiol Behav. 77(4-5):711-6. Review.
Moore BB, Hogaboam CM. 2007. Murine models of pulmonary fibrosis. Am J Physiol Lung Cell Mol Physiol. 2008;294(2):L152-60.
Mossman BT, Churg A. 1998.Mechanisms in the pathogenesis of asbestosis and silicosis. Am J Respir Crit Care Med.;157(5 Pt 1):1666-80.
Nandi S, Burnase N, Barapatre A, Gulhane P, Dhatrak S. 2018. Assessment of silicosis awareness among stone mine workers of Rajasthan state. Indian J Occup Environ Med 22:97-100.
Nelson G. 2013. Occupational respiratory diseases in the South African mining industry. Glob Health Action. 24;6:19520.
Nigam SK, Saiyed HN, Malaviya R, Suthar AM, Desai UM, Venkaiah K, Sharma YK,Kashyap SK. 1990. Role of circulating immune complexes in the immunopathogenesis of silicosis. Toxicol Lett. 51(3):315-20.
Nurwidya F, Damayanti T, Yunus F. 2016. The Role of Innate and Adaptive Immune Cells in the Immunopathogenesis of Chronic Obstructive Pulmonary Disease. Tuberc Respir Dis (Seoul). 79(1):5-13.
Palmer BC, Jatana S, Phelan-Dickinson SJ, DeLouise LA. 2019 Amorphous silicon dioxide nanoparticles modulate immune responses in a model of allergic contact dermatitis. Sci Rep. 25;9(1):5085.
S.Divya priya1 , Dr.S.Suja2, S.Sharmila. 2011. Immunoglobulin classes (IgG, IgA, IgM and IgE) and liver function tests in workers occupationally exposed to cement dust Jr of Pharmacy Research Vol.4.Issue 10. October
Traboulsi H, Guerrina N, Iu M, Maysinger D, Ariya P, Baglole CJ. 2017. Inhaled Pollutants: The Molecular Scene behind Respiratory and Systemic Diseases Associated with Ultrafine Particulate Matter. Int J Mol Sci. 24;18(2).
Watanabe S, Shirakami A, Takeichi T, Ohara T, Saito S. 1987. Alterations in lymphocyte subsets and serum immunoglobulin levels in patients with silicosis. J Clin Lab Immunol. 23(1):45-51.
White ES. 2015. Lung extracellular matrix and fibroblast function. Ann Am Thorac Soc. 12 Suppl 1:S30-3.
Ziskind M, Jones RN, Weill H. 1976. Silicosis. Am Rev Respir Dis. 113(5):643-65.
Zone JJ, Rom WN. 1985. Circulating immune complexes in asbestos workers. Environ Res. Aug;37(2):383-9.

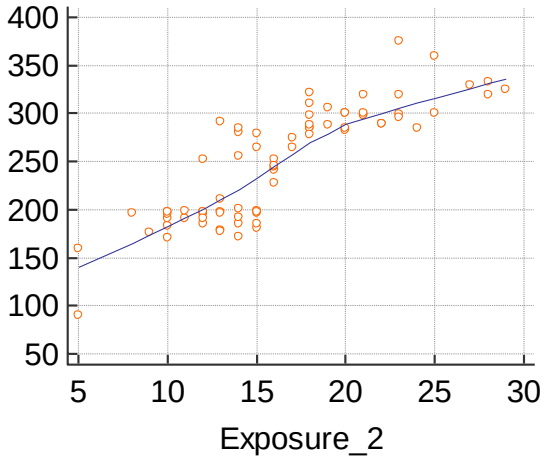

Figure 1. The scatter plot shows positive relationship between exposure period and levels of immunoglobulins IgA)

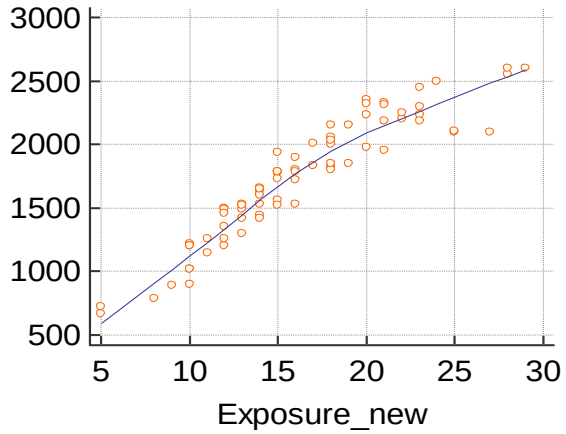

Figure-2. The scatter plot shows positive relationship between exposure period and levels of immunoglobulins IgG

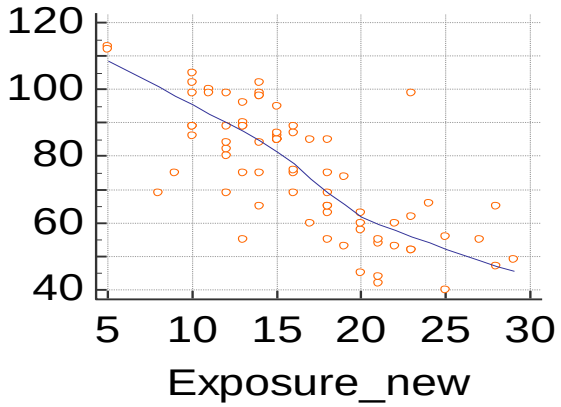

Figure 3: The scatter plot shows positive relationship between exposure period and levels of immunoglobulins IgM

Table

Table 1*:* Descriptive statistics for demographic and behavioral parameters according to study groups

Parameter	**Study group(n=150)**	
	Control (n=75)	Experimental (n=75)
Age (yrs.)	41.58± 8.63	41.88±7.43
Exposure (yrs.)		15.8 ± 4.80
BMI (kg/m2)	26.14±5.20	22.50± 4.64
Diet		
Vegetarian	20 (27%)	14(19%)
Both	57(73%)	61(81%)
Smoking (Yes)	19(25%)	36(48%)
Tobacco (Yes)	27(36%)	44(58.7%)
Alcohol (Yes)	27(36%)	49(65.3%)
Systolic BP	112.5467±10.0004	129.84±15.6249
Diastolic BP	74.44±5.0571	80.48±9.7833

Table 2 Serum immunoglobulin concentrations of silica Exposed workers and Healthy controls (Values are mean ± SE)

Immunoglobulin (mg/dl)	**Control (n=75)**	**Experimental (n=75)**	***P***
IgG	1005.173± 219.5697	1737.187 ±469.6297	P<0.0001
IgA	124.2667 ±30.1126	248.1467 ±58.2065	P<0.0001
IgM	109.4933±19.8613	75.8133±18.5281	P<0.0001