Original Article

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Biotechnology Section

Expression of Low Molecular Mass Proteins in Stone Quarry Workers

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ABSTRACT

Background: In India, there are many unauthorized stone quarries in which millions of workers exposed to crystalline silica dust. Silica dust is the most significant causative agent for the irreversible but preventable disease like silicosis. It is an unavoidable occupational disease among mining population. Silicosis, silicotuberculosis, chronic bronchitis, Chronic Obstructive Pulmonary Disease (COPD) and lung cancer are reported in long term occupational exposure to silica dust. Early health care is the key to silicosis prevention, but until now no effective indicators of early health care is developed.

Objectives: The present study was performed on stone quarry workers for the evaluation of protein fractions by SDS-PAGE electrophoresis.

Materials and Methods: For this study, Stone quarry (n=30) workers were selected from the Panchgaon- kuhifata located in central India as experimental subjects with an exposure of 0-35 years. For comparison, control subjects (n=20) belonging to the same socio-economic status and age group were selected. Blood samples were collected. Separated serum samples were used for SDS-PAGE.

Results: The molecular mass of differentially expressed protein was determined by log graph method which was found to be 10 kDa. Remarkably 90% expression of 10 kDa protein were observed in control subjects but no such expression were determined in serum of stone quarry workers.

Conclusions: The loss of expression of targeted (10 kDa) protein in stone quarry workers may be used as a potential peripheral marker for early silica toxicity.

Key Words: Biomarker, Clara cell protein (CC16), SDS-PAGE, Stone quarry

INTRODUCTION

Each year 17 million workers face respiratory problems due to inhalation of Respirable Crystalline Silica (RCS). Due to poor reporting and uncertain number of exposed individuals, information on the exact number of persons with silicosis is limited. About 10 millions of workers in India are at the risk of Silica dust exposure. Reliable statistical data is not available but there are many industries were exposure to silica dust is known to exist.

Approximately 83% of stone quarry workers are exposed to the respirable silica particles due to different occupations. Similarly, more than one lakh workers encountered high risk of silica dust exposure through mining operation. A sizable proportion of workers have high exposure to respirable silica dust during crushing, blasting, drilling, tunnelling in the stone quarries [1, 2].

Chemically mine dust contains many minerals including Silica, Manganese, Iron, Aluminium and other trace elements but the most prevalent among these, is Silica, which is present almost 70% of the earth crust. Silica is the most common occupational hazardous toxicant for the workers. In 1996 International Agency for Research on Cancer (IARC) classified silica as carcinogen to human beings [3]. Respirable Crystalline Silica (RCS) reduces the lungs ability to extract oxygen from the air [3] and thus leads to many respiratory diseases such as silicosis, silicotuberculosis and Chronic Obstructive Pulmonary Disease (COPD) [4].

Research was carried out on several biomarkers of silicosis. Protein biomarkers with high molecular mass includes Elastase, Angiotensin Converting Enzyme (ACE), KL-6 (Krebs von den Lungen-6), Tumour Necrosis Factor (TNF), Ym protein, HO-1, apoptotic protein (caspase, Fas/FasL) and Heat Shock Proteins 10 (HSP-10), Interleukine 1b- converting Enzyme (ICE), Calcium binding proteins, Clara cell protein

(CC16), neopterin as low molecular mass proteins.

Proteomics strategies were used to identify disease-specific protein markers that could provide the basis for the development for new diagnosis methodologies and early disease detection by differential expression of proteins. The present study aims to focus on the evaluation of protein biomarkers among silica exposed workers from stone quarries for pre-diagnosis of diseases.

MATERIALS AND METHODS

The stone quarries from the Panchgaon-kuhifata located in central India were selected for the study.

Inclusion Criteria

Stone quarry (n=30) workers were selected for this study belonging to the age group 20-60 years and their duration of exposure in the stone quarry were from 0-35 years. For comparison control subjects (n=20) belonging to the same socio-economic status and age group were selected.

Exclusion Criteria

From experimental populations workers working in stone quarry from two-three months were excluded.

A standard questionnaire including health status, family history, dietary habits and duration of exposure were filled and consent form was signed. Blood samples were collected and serum was separated and stored at -40°C.

The samples were distributed in seven groups according to years of exposure of experimental subjects [group I (1-5), group II (6-10), group III (11-15), group IV (16-20), group V (21-25), group VI (26-30) and group VII (31-35)].

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis: SDS-PAGE is a versatile and noteworthy technique widely used for protein separation based on their molecular mass and charges [5]. In this study protein fractions were studied by SDS-PAGE due to its importance in the resolution of serum proteins. The purpose of performing SDS-PAGE was to find a differential expression of proteins among experimental and control subjects.

The standard protein markers were procured from GeNei (Cat. No.-105975). SDS-PAGE was done on a discontinuous vertical thick-layer with a resolving gel of 5% acrylamide in Tris-HCl buffer at pH 6.7 and a separating gel of 10% acrylamide in Tris-HCl buffer at pH 8.9. The current used for resolving gel and for separating gel were 25 mA and 30 mA respectively. Serum sample and markers were mixed separately with sample buffer, containing 4% SDS, 2% 2-mercaptoethanol, glycerol, 0.02% bromophenol blue (tracking dye), and 0.01 M Tris-HCl buffer at pH 6.8. The mixtures were heated in a boiling water bath for 3 minutes to denature the proteins. Serum samples (40 µg per lane) were loaded in each well. After electrophoresis, the gel was stained with 0.05% Coomassie Brilliant Blue R-250 stain which was then destained and fixed by using fixative [6]. The Relative Migration Distance (Rf) was determined using Gel image analyser software and molecular mass of the differentially expressed protein was determined using log graph method.

STATISTICAL ANALYSIS

Statistical analysis was done by using Graph-pad software. Mean ± SD value was calculated. Student's "t" test was used to calculate the significance between Experimental and Control subjects.

RESULTS

The average age among experimental subjects was 31.3 years while in control subjects it was 30.3 years. The average duration of exposure in stone quarry among experimental subjects was 9.5 years. The average smokers were found 42.8% among experimental subjects and 40% in control subjects while 78.5% population were alcoholic in case of experimental subjects and 60% were in control subjects.

The BMI was calculated for both experimental and control subjects. Experimental subjects as compared to control showed 60% population were underweight, while 40% were normal. This may be due to their dietary habits as they belong to Below Poverty Line (BPL) or may be due to family history.

The molecular mass of unknown protein was determined by captivating standard molecular mass markers as reference as shown in [Table/Fig-1]. A standard curve for log molecular mass versus relative migration distance (Rf), was generated using the Precision Plus Protein standards as shown in [Table/Fig-2]. The strong linear relationship ($R2 > 0.959$) between the proteins molecular mass versus relative migration distance, demonstrates exceptional reliability in predicting differentially expressed proteins molecular mass [7].

The molecular mass of the differentially expressed protein was calculated by using the formula:

$y = -2.0355x + 2.3661$ (equation generated from log graph)

x = Rf of differentially expressed Protein = 0.67 cm

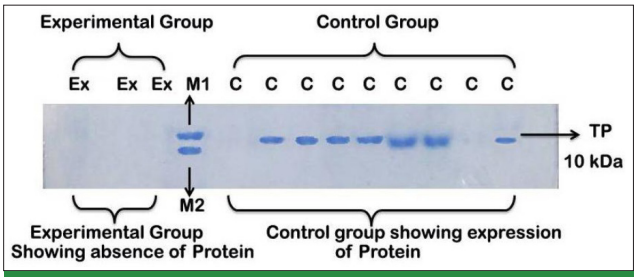
By putting the value of x in above formula,

$y = -1.0025$

Molecular Mass = antilog10y = antilog10-1.0025 = 10 kDa

Molecular Mass of differentially expressed protein = 10 kDa [Table/Fig-1] and [Table/Fig-2].

M1 : Lysozyme (14.3 kDa) Marker



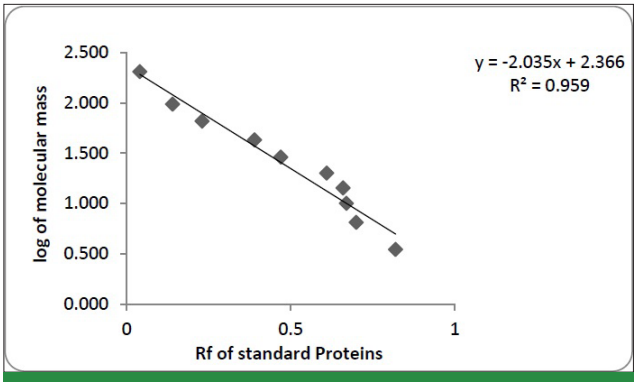
[Table/Fig-1]: SDS-PAGE Gel: Expression of Targeted protein (10kDa) in control group.

M2 : Aprotinin (6.5 kDa) Marker

Ex : Experimental

C : Control

TP : Targeted Protein



[Table/Fig-2]: Molecular Weight determination of targeted protein.

A result showed molecular mass of differentially expressed protein was 10 kDa. Loss of 10 kDa protein fractions was observed in Experimental subjects; on the contrary these proteins were normally expressed in control group. The results on the repeated performance of this experiment showed complete absence (100%) of differentially expressed protein amongst experimental subjects, while expression of same protein was not observed in 10% of control subjects. Remaining 90% control samples showed remarkable expression of the same protein.

DISCUSSION

Several biomarkers were studied for the silica exposed workers. An elevated level of HSP10 and ICE was reported in silica exposed workers at the same time decreased levels of CC16 were documented by Bernald et al., [8] HSP 10 is a regulatory protein, synthesized in the body to protect from stress conditions, which act as an anti-autoimmunity protein helps from the protection of silico-toxicity. ICE is a cytoplasmic cysteine protease, up-regulated by silica particles, plays a key role of modulation of apoptosis. It is a heterodimer of 20-kDa and 10-kDa form.

Results obtained from the SDS-PAGE showed differentially expressed protein in control and exposed population. Silica exposed population showed loss of 10 kDa proteins in experimental population. Significantly reduced levels of CC16 were reported in silica exposed workers. CC16 protein is a homodimer consisting of 70 amino acid subunits, has molecular weight 15.8 kDa. The protein was previously referred as CC10 because of underestimation by SDS electrophoresis [8]. It is potentially immunosuppressive protein, which protects the bronchioles from tissue injury caused by activation of the immune system. Our data implies absence of differentially expressed proteins in 100% of experimental subjects and expression of protein in those workers (100%) who were excluded from experimental subjects.The molecular mass of differentially expressed protein resembles with molecular mass of CC (16). There are two mechanisms by which the concentration of CC16 gets decreased. Primarily silica particles directly damages to Clara cells and decreases its secretion and secondly by Clara cell get directly damaged by activated macrophages (which engulfs silica particles), by releasing cytotoxic mediators [9-11]. From this study it can be predicted that, the protein bands which were absent in the experimental subjects but present in control subjects may be CC 16.

CONCLUSION

In silica-exposed workers some proteins may be change in response to silica and these changes may be used to distinguish silica-exposed populations from the control with the proper discriminate analytical method. The differentially expressed protein having molecular mass 10kDa, may be Clara cell 16, as it has been reported in previous studies that decreased level of this protein have been estimated in silica exposed workers. On the contrary HSP10 and ICE were reported for elevated levels in exposed workers which were not expressed in SDS-PAGE in stone quarry workers.

The finding of protein loss amongst experimental subjects may be used as a biomarker of early toxicity of the lungs' tissue and also in the pre-detection of diseases associated with silica dust exposure.

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