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Asbestos Induced p53 Gene Mutation in Indian Population

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Abstract

Introduction: Asbestos fiber causes gene mutation by formation of reactive oxygen species. Some studies have observed the malignant transformation of tumor suppressor p53 gene by asbestos fibers. Though not much information related to Indian sub population is available.

Material and Method: A total sixty sputum samples were collected from workers in asbestos industries. p53 gene mutation was studied in exon-5 and exon-7. The DNA was extracted from sputum samples and PCR-SSCP analysis was done by routine procedure.

Results: In p53 gene exon-5, 3.33% mutation was observed.

Discussion: The mutagenic nature of asbestos fiber has been reported by many researchers. We also observed 3.33% mutation in exone-5 of p53 gene in sputum samples of Indian asbestos factory workers. However some ambiguous results are also available. Kitamura et al did not observe any mutation in p53 gene exons 5 - 9, in patients of mesothelioma exposed to asbestos.

Conclusion: The carcinogenic changes caused by asbestos fibers needs further investigations.

Key words: carcinogen, oncogenes, mutations, asbestos

Introduction:

Asbestos is a naturally occurring hydrated mineral silicate and are resistant to heat, fire and corrosion. It is used in manufacturing of construction material, heat resistant fabric, automotive and shipbuilding industry. [1] Although asbestos is extensively used, its unsafe effects on human health have been recognized since 1899.[2] The International Agency for Research on Cancer in 1977 declared asbestos as Group-1 carcinogen.[3] Many countries have banned the asbestos, however India has not reduced its usage in spite of its hazardous effects. [1]

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Exposures to all forms of asbestos are significant occupational and environmental risk factor for cancer.[4] The airborne asbestos particles when inhaled can become trapped in the lung tissue and can enhance the possibility of cancer development.[5] Also other organs such as gastrointestinal tract, larynx, kidney, liver, pancreas, ovary, and hematopoietic systems show a high prevalence in asbestos induced cancer.[6-7] The latency of cancer development is thirty to forty years after the asbestos exposure, it will continue to cause significant disease morbidity and mortality in years to come.[8]

Asbestos fibers cause genetic and cellular injuries, which may further cause malignant transformation of lung cells.[9] Some studies suggest that, asbestos, silicon fibers are internalized by alveolar epithelial cells, resulting in the production of reactive oxygen species (ROS), DNA damage, and apoptosis by activating p53 gene.[10] The asbestos has potential to alter the activity of tumor suppressor genes. The altered role of p53 gene in pathophysiology of asbestos-associated malignancies has been extensively reported.[11] These data suggest that p53 gene has an important pathophysiological role in regulating the lung epithelial cell DNA damage response, after the exposure to asbestos. [12]

The presence of asbestos bodies in sputum samples is suggestive of significant occupational asbestos exposure. Sputum is easily accessible biological fluid that possibly contains tumor-cell from upper and lower respiratory tract. Some studies have detected mutations of tumor-suppressor gene p53 and K-Ras by molecular analysis of sputum. [13] The collection of sputum sample is simple, less invasive, reliable diagnostic tool for the early diagnosis of lung cancer. [14] The study of p53 gene mutation in asbestos exposed patients has generally being done on lung biopsies. Fewer studies are available for sputum samples molecular level research in asbestos exposed Indian workers. We collected sputum samples of workers from industries using asbestos and analyzed it for the study of p53 point mutation in Indian sub population.

Material and Method

This study was undertaken in Maulana Azad Medical College-New Delhi, Department of Pathology and Indian Institute of Cytology and Preventive Oncology.

In total sixty sputum samples were collected from workers working in car break, insulation and cement sheet manufacturing industries using especially chrysotile fiber. All of them had

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exposure of 4-8 yrs to asbestoses fiber. However the DNA could be extracted only from thirty samples due to insufficient sample. Subjects whose sputum samples were analyzed in this study showed a minimum of clinical symptoms. Each individual gave informed consent and also answered a standardized questionnaire on information related to working history, family and personal medical history. All subjects were male (n = 60). Of these individuals, twenty (n=20) had symptoms of chronic bronchitis, with excessive bronchial mucus and a chronic cough for more than three months, rest did not show any symptoms.

Patients rinsed their mouths with water, breathed deeply, held their breath and coughed. The expectorated sputum was collected into a sterile plastic sample container. They were sealed and stored at -80°C till further processing. The sputum samples were kept in 50 ml eppendorff tube with 20 ml DAS fluid (50% ethanol + 2% polyglycol), shaken and stored at 4 degree centigrade. Two to three drops of sputumlysin with 6.5mmol/l dithiothreitol were added to each sample. The samples were vortexed and centrifuged at 1500 rpm, the supernatant was discarded and precipitate was washed with PBS twice. The precipitate was transferred to 1.5 ml eppendorff tube, and genomic DNA was extracted by SDS/proteinase K and phenol-choroform extraction following standard protocol. Normal control DNA was obtained from peripheral lymphocytes of healthy persons.

PCR under optimal conditions was performed in 25 μl of reaction solution, including 10× PCR buffer, 2.5 μM of each specific primer, 25 mM $MgCl_2$, 2.5 mM dNTP, five units of Taq DNA polymerase and 20 ng/μl of DNA products. Amplifications were performed with the following cycling profile: pre-denaturation at 95 ˚C for 5 min followed by 40 cycles of denaturing 95 ◦C for 30 s; annealing for exon 18 at 58.5 ˚C, exon 19 at 57.5 ˚C, exon 20 at 55.5 ˚C and exon 21 at 59.5 ˚C; extension at 72˚C for 1 min and the last cycle was followed by a final extension at 72 ˚C for 10 min. Amplified products were analyzed by agarose gel electrophoresis and ethidium bromide staining. [15-17]

Exon 5 Forward 5´-ATCTGTTCACTTGTGCCCTG-3´ 274 base pair

Reverse 5´-AACCAGCCCTGTCGTCTCTC-3´

Exon 7 Forward 5´-CCAAGGCGCACTGGCCTCATC-3´ 205 base pair

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Reverse 5´-CAGAGGCTGGGGCACAGCAGG-3´

Single-strand conformation polymorphism (SSCP). A single base change in the sequence can cause single-stranded DNA to migrate differently. In this study we investigated p53 mutant genes in exons 5 and 7 of sputum DNA (PCR) samples. The reaction was mixed, spun down and denatured at 97˚C for 10 min. Then the reaction was cooled on ice at -20 o C for 10 min. The ratio of acrylamide:bis for gel preparation was 50:1. The voltage used was 150 V on ice for 150 min. The gel was separated from the gel cassette carefully in water due to the polyacrylamide gel being quite thin. After that, the gel was stained with ethidium bromide and photographed under ultraviolet light. [15- 18]

Results

PCR-SSCP analysis: In sputum analysis, we found all workers sputum was positive for asbestos fibers and some of them had asbestos bodies.

Single-strand conformational polymorphism (SSCP) analysis is a simple and sensitive technique for mutation detection and genotyping. The principle of SSCP analysis is based on the fact that single-stranded DNA has a defined conformation however, altered conformation due to a single base change in the sequence can cause single-stranded DNA to migrate differently under non denaturing electrophoresis conditions. So the wild-type and mutant DNA samples display different band patterns. Thirty samples of sputum DNA for p53 exon 5 and exon 7were subjected to SSCP. Difference in the mobility of band pattern was observed in one sample for exone-5 of p53 gene (3.33%). The exon 7 of p53 gene did not show any mutation.

Fig:1. Difference in the mobility of band pattern was observed in one sample for exone-5 of p53 gene (3.33%).

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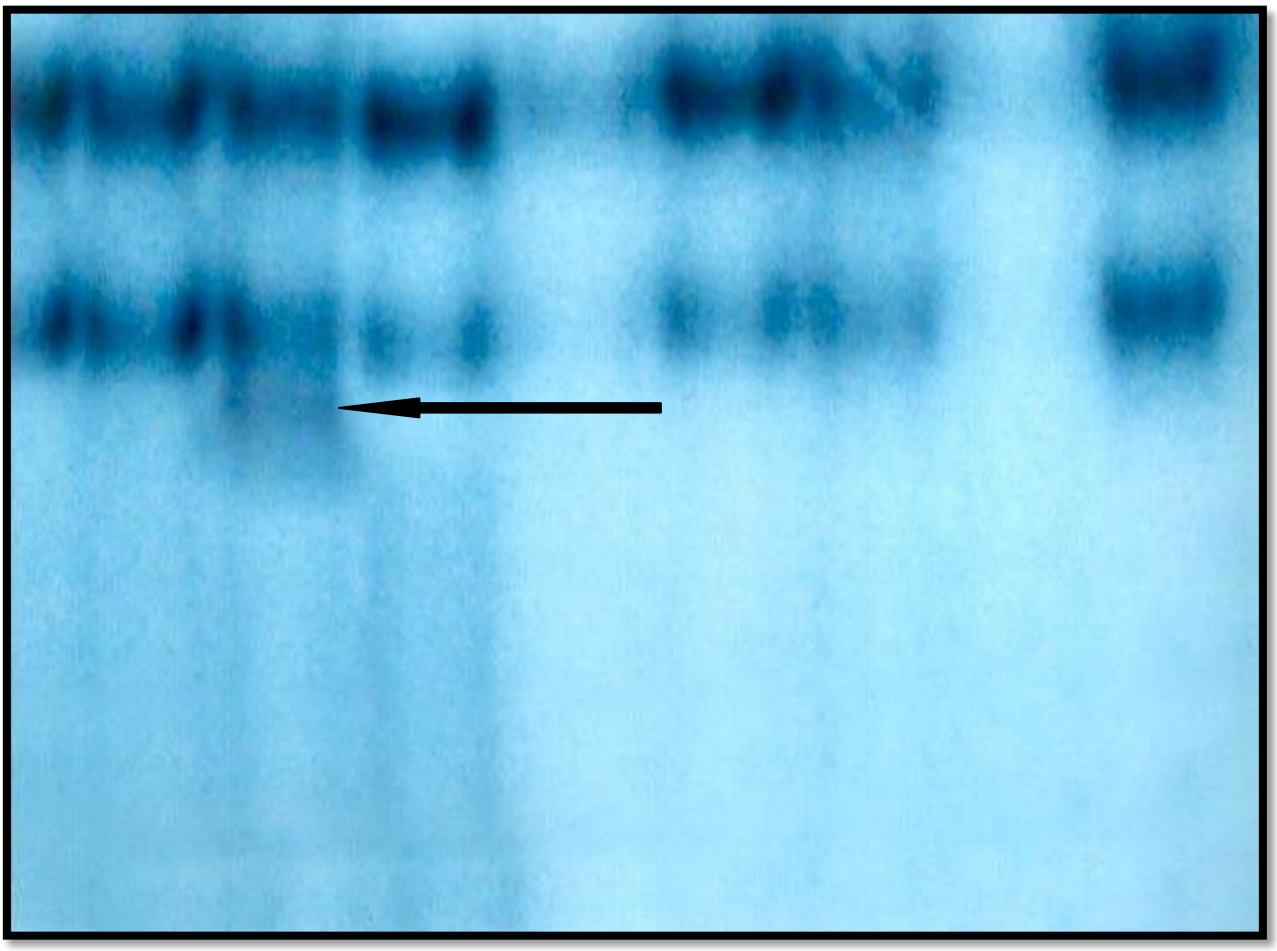

Discussion

The genotoxic nature of asbestos fibers is well documented, they can generate mutagenic and cytotoxic changes at cellular level [19] but exact mechanism by which asbestos induce molecular changes during pulmonary carcinogenesis are not clear. [20]

The likely exposer to asbestos is through inhalation of airborne fibers. The fibers of chrysotile, amosite and crocidolite, are retained in the upper air ways (nose, throat, trachea). Some studies

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have proposed that asbestos causes pulmonary toxicity by generating reactive oxygen species that regulates the DNA damage response by inducing genetic modifications in the cells, which in turn activates p53 gene. It causes cell cycle arrest and initiates DNA repair and, if DNA damage is extensive, augment apoptosis by the mitochondria-regulated death pathway. Thus prevents mutations from accumulating. [21]

Altered p53 expression is implicated in the pathophysiology of asbestos-associated malignancies. [22] The research data related to carcinogenic effect of asbestos for Indian sub population is very less. We decided to examine p53 gene mutation in Indian factory workers exposed to asbestos fibers and studied p53 gene mutation. We observed 3.33 % gene mutation in exon-5 of p53 gene.

Some reports suggest Crocidolite asbestos induces p53 gene mutations, predominantly in exon 9.[23] Further, increase in p53 protein levels in lung cancer of patients with asbestosis is observed by some researchers. [24] Some findings also suggest the increased occurrence of p53 mutations in patients with occupational history of asbestos exposure, (5%) for patients without p53 mutations versus (20%) of those with p53 mutations, ($P < 0.05$) [25]. Liu et al studied ten cases of asbestos associated cancer, seven cases were found to have mutations by PCR-SSCP in exon-8 of p53 gene. [24] Collectively, the data suggest the important role of p53 gene in regulating the lung epithelial cell DNA damage response after exposure to asbestos.

Some studies with contradictory results are also available in research literature. Kitamura et al observed no mutations in the p53 gene exons 5 - 9, in malignant mesothelioma patients exposed to asbestos.[26] Another study conclusion suggested that asbestos exposure is probably not related to the increase of p53 protein level and, the frequency of p53 gene mutation in mesotheliomas. [27] Some studies suggest that malignant transformation of human mesothelioma cells do not require inactivation of p53 gene by point mutations. The frequency of p53 mutations was 39% among the cases with occupational asbestos exposure, as compared with 54% among the non exposed cases; the difference was not significant. [28] Another study had similar conclusion and observed that the group with the heaviest occupational exposure appeared to have a lower prevalence of p53 mutations (20%) than the patients with lower asbestos fiber counts (48%). The results suggested a negative correlation between pulmonary asbestos fiber burden and p53 mutation frequency. [29]

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May be the mutational spectrum of asbestos associated cancers, might be different from that of non-asbestos associated cancers.

Epidemiological and experimental studies have established that asbestos is a carcinogen as well as co-carcinogen.[2-3] Enormity of danger has compelled many countries to ban asbestos and others have limited its use, [30] however India still import, utilizes, and process asbestos. Thus ill effect of exposures to asbestos remains unchecked in India.[31] Although it is mandatory to report asbestosis and asbestos-related cancers as per the subsection-1 of Section 25 of the Mines Act, 1952. However, no ARDs were reported as per the 12th 5-year plan document of the government. [32] This has the potential to put the life of workers in the processing plants, and the people exposed indirectly to asbestos fibers, at risk. It is time for Indian government to review its policies related to asbestos use seriously. Since India is a developing country in future it will be difficult to bear the burden of the disease.

Reference:

1. Asbestos. In: Indian Minerals Yearbook (Part-III: Mineral Reviews). 56th ed. Government of India, Ministry of Mines, Indian Bureau of Mines; 2017.
2. Lucy D. Report on the Health of Workers in Asbestos and other Dusty Trades in HM Chief Inspector of Factories and Workshops, 1899, Annual Report for 1898. London: Majesty's Stationery Office; 1899. p. 171-2.
3. International Agency for Research on Cancer. Asbestos. IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans. Vol. 14. Lyon, France: International Agency for Research on Cancer; 1977. p. 106.
4. Kanarek MS. Mesothelioma from chrysotile asbestos: Update. Ann Epidemiol 2011;21:688-97.
5. K. O'Byrne and V. Rusch. Malignant Pleural Mesothelioma, Oxford University Press, New York, NY, USA, 2006.
6. J. E. Craighead, "Nonthoracic cancers possibly resulting from asbestos exposure," in Asbestos and Its Diseases, J. E. Craighead and A. R. Gibbs, Eds., pp. 230–252, Oxford University Press, New York, NY, USA, 2008.
7. J. E. Craighead, Al. Gibbs, and F. Pooley, "Mineralogy of asbestos," in Asbestos and Its Diseases, J. E. Craighead and A. R. Gibbs, Eds., pp. 23–38, Oxford University Press, New York, NY, USA, 2008.

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8. Virta R. L. 2003. Worldwide asbestos supply and consumption trends from 1900 to 2000. Reston, VA: U.S. Department of the Interior, U.S. Geological Survey.
9. Barrett J.C. Cellular and molecular mechanisms of asbestos carcinogenicity: Implications for biopersistence. Environ. Health Perspect. 1994;102(suppl. 5):19–23.
10. Kamp DW, Weitzman SA. The molecular basis of asbestos induced lung injury. Thorax. 1999;54:638–652.
11. Green DR, Kroemer G. The pathophysiology of mitochondrial death. Science. 2004;305:626–629.
12. Oren M. Decision making by p53: life, death and cancer. Cell Death Differ. 2003;10:431–442.
13. Belinsky SA, Grimes MJ, Casas E, et al. Predicting gene promoter methylation in non-small-cell lung cancer by evaluating sputum and serum. Br J Cancer. 2007;96(8):1278–1283.
14. Palmisano WA, Divine KK, Saccomanno G. Predicting lung cancer by detecting aberrant promoter methylation in sputum. Cancer Res. 2000;60(21):5954–5958.
15. Mao L, Hruban RH, Boyle JO. Detection of oncogene mutation in sputum precedes diagnosis of lung cencer. Cancer Rec.1994;54:1634-1637.

16. B. Wang, L LI, L YAO, L LIU,Y ZHU. Detection of p53 gene mutation in sputum samples and their implication in the early diagnosis of lung cancer in early diagnosis of lung cancer suspicious patients. Chinese Med Jour.2001;114(7):694-697.
17. Cline J, Braman JC, Hogrefe HH. PCR fidelity of DNA polymerase and other thermostable DNA polymerases. Nucleic Acids Res 1996;24:3546–51.
18. Orita M, Iwahana H, Kanazawa H, Hayashi K, Sekiya T. Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformation polymorphisms. *Proc Natl Acad Sci U S A*, 1989;86: 2766-70.
19. Msiska Z, Pacurari M, Mishra A. DNA double-strand breaks by asbestos, silica, and titanium dioxide: possible biomarker of carcinogenic potential? Am J Respir Cell Mol Biol. 2010;43 (2):210–219.
20. Calvayrac O, Pradines A, Pons E, et al. Molecular biomarkers for lung adenocarcinoma. Eur Respir J. 2017;49(4):1601734.
21. Jones PA, Baylin SB. The fundamental role of epigenetic events in cancer. Nat Rev Genet. 2002;3(6):415–28.
22. Burmeister B, Schwerdtle T, Poser I. Effects of asbestos on initiation of DNA damage, induction of DNA-strand breaks, P53-expression and apoptosis in primary, SV40-transformed and malignant human mesothelial cells. Mutat Res. 2004;558(1–2):81–92.
23. K Unfried, N Kociok, M Roller J Friemann, F Pott, W Dehnen P53 mutations in tumours induced by intraperitoneal injection of crocidolite asbestose and benzol [a]pyrene in rats. Exp Toxicol Pathol. 1997;49(3-4)181-7.
24. B C Liu , D C Fu, Q Miao, H H Wang, B R You. p53 gene mutations in asbestos associated cancers. Biomed Environ Sci. 1998;11(3):226-32
25. Wang X, D. C Christiani, J. K Wiencke, M. Fischbein, X, Xu T, Cheng E. Mark, J. C. Wain, and K. T. Kelsey. Mutations in the p53 gene in lung cancer are associated with cigarette smoking and asbestos exposure. Cancer Epidemiol. Biomarkers Prev. 1995;4:543–548.
26. Kitamura F', Araki S, Tanigawa T', Miura H, Akabane H and Iwasaki R. Assessment of Mutations of Ha- and Ki-ras Oncogenes and the p53 Suppressor Gene in Seven Malignant Mesothelioma Patients Exposed to Asbestos-PCR-SSCP and Sequencing Analyses of Paraffin-Embedded Primary Tumors. Industrial Health 1998; 36: 52-56.

ISSN: 0975-3583, 0976-2833 VOL14, ISSUE 08, 2023

27. Nuorva K R. Mäkitaro E. Huhti, D. Kamel K. Vähäkangas R. Bloigu Y. Soini and P. Pääkkö. p53 protein accumulation in lung carcinomas of patients exposed to asbestos and tobacco smoke. Am. J. Respir. Crit. Care Med. 1994;150:528–533.
28. Pursiainen K H, Karjalainen A, Kannio A, Anttila S, Partanen T, Ojajärvi A , and Vainio H. Lung Cancer and Past Occupational Exposure to Asbestos Role of p53 and K-ras Mutations . Am. J. Respir. Cell Mol. Biol. 1999; 20: 667–674.
29. Harty L C, D. G. Guinee, Jr, W. D. Travis, W. P. Bennett, J. Jett, T. V. Colby, H. Tazelaar, V. Trastek, P. Pairolero, L. A. Liotta, C. C. Harris, and N. E. Caporaso. P53 mutations and occupational exposures in a surgical series of lung cancers. Cancer Epidemiol. Biomarkers Prev. 1996;5:997– 1003.
30. WHO Report. Asbestos-Economic Assessment of Bans and Declining Production and Consumption;2017.Availablefrom:http://www.euro.who.int/_data/assets/pdf_file/0009/341757/341757/Asbestos_EN_WEB_ reduced.pdf. [Last accessed on 2018 Apr 22].
31. Le GV, Takahashi K, Park EK, Delgermaa V, Oak C, Qureshi AM, et al. Asbestos use and asbestos-related diseases in Asia: Past, present and future. Respirology 2011;16:767-75.
32. Report of the Working Group on Occupational Safety and Health for the Twelfth Five Year Plan (2012 TO 2017); Government of India, Ministry of Labour and Employment; 2011. Available from: https://www.ilo. org/wcmsp5/groups/public/-ed_protect/-protrav/-safework/documents/ policy/wcms_211795.pdf.