Original Research Paper

Volume-8 | Issue-12 | December-2018 | PRINT ISSN No 2249-555X

Zoology

INCIDENCE OF MICRONUCLEI IN BUCCAL EPITHELIAL CELLS OF COAL MINERS

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ABSTRACT Coal is known carcinogen. Workers employed in coal industry are exposed to larger amounts of cement dust particulates in work place results to harmful health effects. In the present study assessment of genetic damage was evaluated using micronucleus assay in coal industry workers buccal epithelial cells. A total of 80 subjects were selected from Singareni collaries kothagudem, Telangana, India and 60 individuals were served as controls having same diet, social economic background. The buccals were collected from oral cavity and analysed for nuclear abnormalities. A high frequency of karyolysis was noted in exposed group with longe duration of exposure. A significant increase in the frequency of micronucleus was observed in exposed group when compared with control data. A synergistic effect was observed in smokers exposed group. Thus the data clearly indicate the effect of coal dust in coal industry workers, the present studies indicate the use of micronucleus assay to identify the the groups that are risk for developing cancer.

KEYWORDS : Coal, micronuclei, buccal cells

INTRODUCTION:

Introduction Coal is one of the most abundant minerals in nature and constitute the largest fossil fuel source used for the generation of energy (León-Mejía et al. 2011). Coal production is prominent in various countries, e.g. the Russian Federation, Brazil, South Africa, Australia and others. Working in coal mines, despite all achievements of modern science and technology, still remains among the most dangerous occupations to the health. Coal-mining is characterised by long-term contact with various harmful occupational agents, such as coal dust, (polycyclic aromatic hydrocarbons (PAC), ionising radiation, dampness, din and heavy metals. The influence of such agents leads not only to an increase in the risk of initiation of various chronic cardiovascular diseases, nervous diseases (vibrational angioneurosis, sensorineural hearing loss and muscle-toning syndrome) and pulmonary diseases [chronic dust-induced bronchitis (CDB), coal-workers' pneumoconiosis (CWP) and lung cancer] but also to genotoxic risk [Rohr et al, 2013]. Genotoxic risk is caused by the exposure to PAH, heavy metals and ionising radiation. DNA damage resulting from exposure to genotoxic agents can be registered at the chromosomal level using methods of assessing chromosomal aberrations in human peripheral blood lymphocytes [Vijender Reddy et al, 1994; Vijender Reddy and Rudrama Devi, 1996; Snigiryova et al, 2011]. In the present investigation studies were carried out in coal miners using micronucleus assay in buccal epithelial cells.

MATERIALS AND METHODS:

Subject Recruitment and Sample Collection:

The study was conducted on 80 workers aged 16- 60 years from Telangana region of india.. The control groups consisted 60 people, aged 16-66 with no history of exposure to clastogenic and/or aneugenic agents and socio-economic level also similar to that of experimental subjects. At the time of sample collection all the coal workers signed a term of informed consent and replied to Questionnaires elaborated to determine the profile and habits of study population. The protocol has been approved by local ethical committee. The exposed workers to coal the duration of service was taken more than five years. The study was carried out in 80 coal exposed workers. The control group consists of 60 healthy individuals with no exposure to any toxicant or any other chemicals participants are informed about the study, asked to sign the consent form and complete the questionnaires to obtain necessary information ion their life style and personal habits (age, working duration smoking habits, health etc.)

Analysis of micronucleus assay:

Prior to buccal cell collection the tannery workers were advised to rinse their mouth thoroughly with water to remove unwanted debris. Sterile wooden spatula was used to obtain cell samples from buccal mucosa. The mucosa was transfer to Eppendff of tubes with PBS at Ph. 7.0 and centrifused for 10 min at 1500 rpm. Supernatant was removed and replaced with fresh PBS solution. This process was repeated thrice and the pellet was smeared on clean slides. Smears were air dried and fixed in 1:3 acetic acid and methanol fixative for 10 min. slides are air dried and stained with 2% Giemsa for 10 Min. the slides are observed under microscope. Scorning criteria for buccal cytome assay from each sample threeslides were scored Nuclear abnormalities were classified according to the Tolbert et. al. [1992). These criteria are intended to classify buccal cells into categories that distinguish between "Normal and Abnormal" based on their aberrant nuclear morphology. The abnormal morphologies are due to the DNA damage and cell death.

Scoring method and statistical analysis: To determine the frequency of vaiorus cell types, about 1000 cells were scored for the presence of micronuclei cell, binucleared cells, karryoorhectic and karyoloytic ells. All the data were expressed a the Mean Standard Deviation. The synergistic effect between smoking and exposure were tested with a two way analysis of variance. Multiple comparisons were made by using a least significant difference test. The error rate was accepted as 0.05 by student + test.

RESULTS

Table 1 and 2 shows the main characteristics in controls cases studies. The mean age group of the selected workers belongs to the range from 31.6 ± 4.1 to 41.2 ± 6.0 in control group and from 36.0 ± 5.01 and 42.0 ± 7.14 in the exposed group they belonged to the similar social economic status. The characteristics of the studied group are mentioned in Table 1. The cytological observations reveals micronuclei and binucleated cells of buccal smears. The mean value of micronuclei in smokers was 8.40 ± 1.66 as against 5.72 ± 1.43 in non smoker exposed group. The mean value of binucleated cells in subjects without smoking was 9.20 ± 0.68 as against 12.12 ± 1.04* in subjects with a habit of smoking higher cells of Karyorrhix cells (KRC). The values were significant higher in smoker of exposed subjects compared to non smokers exposed group. This indicating this habit of smoking enhanced the man values of KRC and KLC nuclear anomalies when compared to control values. The frequency of micornucleate cells, binucleate cells, Karyohexis and Karyolysis cells were compared in duration of exposure less than 5 years and in ten years exposure and it more significantly higher in ten years of service workers

Table 1: Demographic characteristics of study subjects

Group	No. of subjects	Age in years	Duration of exposure
Control	60	43.33±2.3	19.5±1.8
Exposed	80	42.52±2.8	18.8±1.91
Non smokers	45	41.11±4.01	19.5±1.27
Smokers	35	42.83±5.01	18.3±2.60

Table 2: Cytological observations in control and exposed group

Individuals	MNC	BNC	KRC	KLC
Control	3.68 ± 0.46	4.60 ± 0.82	9.20 ± 0.68	30.20 ±0.42
Smokers (N=28)	2.28 ± 0.12	2.60 ± 0.18	7.20 ± 1.80	22.12 ±1.62
Non Smokers (N=32)				

Exposed Smokers (N=35) Non Smokers (N=45)	8.40 ± 1.66* 5.72 ± 1.43	7.82 ± 1.01* 5.62 ±1.06	12.12 ± 1.04* 7.42 ± 6..62	42.32 ± 1.06* 31.12 ± 1.10
Duration of exposure 5 years (N = 35) 10 years (N = 45)	4.82 ± 1.62 9.40 ± 0.60	4.24 ± 0.28 10.20 ± 1.80	16.0 ± 1.66 22.0 ± 1.48	3.40 ± 1.28 42.0 ± 1..96

*P<0.05

DISCUSSION:

Micronucleus test of exfoliated cells in epithelial tissue have been used to evaluate the genotoxic effects. Micronucleus is defined as microscopically visible, round or oval cytoplasmic chromatin mass next to the nucleus. Micronuclei originated from aberrant mitosis and consist of acentric chromosomes, chromatid fragments or whole chromosomes that have failed to be incorporated in to the daughter nuclei during mitosis. Micronucleus test is the most frequent technique used to detect chromosome breakage or mitotic interference associated events with increased risk for cancer[Curtis 1982]. As micronuclei derive from chromosomal fragment and whole chromosomes lagging behind in anaphase, the micronucleus assay can be used to show both clastogenic and aneugenic effects. Micronucleus formation is undoubtedly an important mechanism for chromosome loss [Ford1992]. service workers. constitute a challenge. There is an increased risk of initiation of pulmonary and nervous diseases, as well as various personal injuries, coal miners are exposed to genotoxic risk resulting from the influence of chemical and physical agents on the organism.

Cytogenetic tests permitting the detection of various types of DNA damage at the cellular and chromosomal level can be used for the assessment of such genotoxic risk. Currently, many cytogenetic tests are used for the investigation of coal miners. A number of articles describe the increased level of micronuclei (MN) and other cytogenetic abnormalities discovered using the cytokinesis-block micronucleus assay on peripheral bloo lymphocytes [León-Mejía et al, 2011; Rohr et al, 2013; Donbak et al, 2005], the micronucleus assay on exfoliated cells [Rohr et al, 2013; Kumar et al, 2011; Kvitko et al, 2012] and DNA damage discovered using the DNA-comet assay [León-Mejía et al, 2011; Rohr et al, 2013] in coal-miners in comparison with people who do not work in coal mines. Donbak et al. [2005] used the sister chromatid exchange (SCE) test and discovered an increased level of this marker in coal miners from Turkey. Thus, we can say that miners are characterised by an increased level of cytogenetic damages associated with a considerable genotoxic influence rations per 100 cells was very similar to results—5.82 ± 0.87%) Earlier we have reported the higher percentages of chromosomal aberrations and sister chromatid exchanges in coal miners Andhra Pradesh when compared with the control data. [Vijender Reddy et al, 1994, Vijender Reddy and Rudrama devi, 1996].

The micronuclei in exfoliated epithelial cells are useful biomarkers of occupational exposure to genotoxic chemicals. Cigarette smoking is one of the factor that may influence the rate of DNA damage such as incidence of micronuclei in humans [Celik et. al. 2003] reported that cigarette smoking significantly increase the frequencies of nuclear abnormalities in both controls and exposed subjects. Increase in exposure to toxic chemicals such as formaldehyde and benzeze induces a significant increase in the bucall cell micronuclei [Titenko Holland et. al. 1996, Suralles et al. 1997] copper smelters [Lewinska et. al 2007], shoe factory workers [Gian et. al. 2009].

The harmful effect of dust in various forms of human health have been already demonstrated [Gutherie 1992, Dong et al. 2006]. The MN Test scientifically approved is important in demonstrating the genotoxic effects of harmful substance on health [Nerseyan, 2005 Fenech et al. 2007] such as measuring genotoxicity in petrol station employees [Celik, 2003] agricultural workers [Pastor et. al, 2002] Cigarette smokers and tobacco users [Prioita et. al. 2006] workers exposed to pesticides [Pastor et al. 2002] timber dust [Celik and Kanik 2006] Ozone and Cancer patients [Chen et. al. 2006]

CONCLUSIONS:

In conclusion, biomonitoring studies of workers exposed to coal dust are rather vague because each population has a different life style factors but same occupation in different areas under different climatic and environmental conditions and are exposed to indistinguishable mutagen. Since DNA damage is an important step in events leading from carcinogen exposure to cancer diseases and subsequently the results of the present study have an impact on coal miner's progeny. Therefore, there is a need to educate those who work in coal mine industry about the potential hazard of occupational exposure and the importance of using protective measures.

ACKNOWLEDGEMENT

The author (KRD) thankful to University authorities and to Prof. V. Krishna Reddy, Head, Department of Zoology, Osmania University, Hyderabad for providing necessary laboratory facilities.

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