Original Article

The adverse effects of mining pollutants on oral mucosa in Bellary district: A clinical and genotoxicity study

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Abstract

Background: The Bellary district in Karnataka, rich in mineral resources, is a major mining industry, but prolonged exposure to mining can lead to health hazards. The study aims to assess the genotoxic impact of mining pollutants on mine workers using the micro-nucleus (MN) assay.

Setting and Design: Cross-sectional study.

Materials and Methods: A total of 250 individuals (198 males and 52 females) working in mining areas were examined, and their oral findings were recorded in a proforma. For the micro-nucleus assay, buccal smears from 30 individuals working in mining areas with habits, 30 individuals working in mining areas without habits, and 30 individuals residing in non-mining areas (control group) were selected. Smears were stained with Giemsa stain to identity and quantify the MNs.

Results: The frequency of oral mucosal problems among 250 persons working in mining regions was 170 (68.0%) with no oral mucosal conditions, 79 (32.6%) with oral mucosal conditions, 25 (10%) with leukoplakia, 1 (0.4%) with lichen planus, and 8 (3.2%) with ulcerations. Acute necrotising gingivitis was reported in one person (0.4%), candidiasis in two (0.8%), abscess in two (0.8%), OSMF in 39 (15.6%), and oral cancer in two (0.8%). The mean MN count was 2.40 + 1.57 in mine employees with habits, 2.18 + 1.25 in mine workers without habits, and 1.40 + 0.55 in normal healthy controls.

Conclusion: Reduced occupational health risks brought on by exposure to mining contaminants require protective measures. After being exposed to mining pollutants, exfoliated buccal mucosal cells can be examined for genotoxicity.

Keywords: Genotoxicity, lesions, micro-nucleus, mining, mucosal, pollutants oral

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INTRODUCTION

Mining is the extraction of precious minerals or other geological elements from the ground via an orebody, lode, vein, seam, or reef, which creates the miner's mineralised package of economic interest. Metals, coal and oil shale, gemstones, limestone and dimension stone, rock salt and potash, gravel, and clay are among the ores extracted through mining. Mining is necessary to extract any

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material that cannot be generated naturally or artificially in a laboratory or factory. In a broader sense, mining covers the exploitation of any non-renewable resource, such as petroleum, natural gas, or even water. Mining was possibly the second of humankind's earliest efforts after agriculture. Early on, the two industries were classed as the primary or fundamental industries. Mining has played an essential role in human history from pre-historic times to the present.[1]

Bellary district is located on the eastern part of Karnataka state and extends from southwest to northeast. This area is rich in mineral resources. The yearly production of iron ore ranges from 2.75 to 4.5 million tonnes, while manganese ore ranges from 0.13 million tonnes to 0.3 million tonnes. The most significant minerals discovered in Bellary mining sites are manganese, iron, lead, copper, zinc, and others. The average suspended particulate matter concentration varies between 130 g/m^3 and 1678 g/m^3 at various mining sites. Even if the concentration is within the authorised limits, it is certain to have a negative impact on the health of the miners and those living around. Long-term mining exposure is associated with a number of health hazards, including an increased prevalence of mouth mucosal ulcers among mine employees.[2]

Furthermore, skeletal anomalies such as rickets were documented among children living in the mining region, suggesting that mining pollutants may have an unfavourable influence on growing teeth.[3]

A micro-nucleus (MN) is a tiny additional nucleus that is detached from the main nucleus during cellular division by late chromosomes or chromosomal fragments. It is a microscopically observable round to oval cytoplasmic chromatin clump in the extra-nuclear region. Numerous genotoxic substances that cause chromosomal damage trigger them in cells. When centric elements travel towards the spindle poles, the damaged chromosomes lag behind in anaphase as acentric chromatids or chromosomal fragments.

Following telophase, the intact chromosomes and central pieces give birth to normal daughter nuclei. The lagging components are also present in the daughter cells, but a significant fraction is changed into one or more subsidiary nuclei, which are typically considerably smaller than the major nucleus and are so referred to as MNs.

Larger MNs originate from the exclusion of a whole chromosome after disruption to the cell's spindle system (aneugenic effect), whereas smaller MNs come from structural abnormalities that cause chromosomal fragments (clastogenic effect).[4]

The buccal cell MN assay was initially proposed in 1983.[5] It is becoming more prominent as a biomarker of genetic damage in a variety of applications. MN assay gives information on cytogenetic damage in tissues that are targets of human carcinogens and from which carcinomas might originate. Oral squamous cell carcinomas have complicated karyotypes that include several chromosomal deletions, translocations, and structural abnormalities.[6]

As the focus shifts to discovering techniques to detect early genotoxic damage in those working in the mining region, the MN test provides a simple and non-invasive yet reliable screening approach for measuring early genotoxic damage far before any clinical or histological symptoms of cancer are obvious. As a result, the purpose of this research is to identify and document oral mucosal lesions as well as to assess the genotoxic effect of mining pollutants on mine workers using the MN test.

MATERIALS AND METHODS

The study analysed the impact of mining pollution on oral soft and hard tissues in 250 individuals working in Bellary mines. The research involved 60 miners with and without tobacco habits and 30 non-mining non-smokers as a control group. The inclusion and exclusion criteria included 30 miners with or without cigarette use history and patients with oral mucosal or systemic illnesses. A study involving 250 miners aged 15–60 was conducted, with each participant given information and consent forms. A comprehensive oral examination was conducted to identify dental abnormalities and lesions.

An MN test was conducted on 60 people, including 30 with and 30 without tobacco-related habits and 30 non-mining employees. Cells were smeared on glass slides, fixed in methanol/acetic acid, and stained with Giemsa stain.

MN was identified on slides, counted in 500 cells, coded, and counted twice by two observers to prevent bias and overlapping cells. The process was coded to reduce overlapping and overlapping cells.

Results were analysed after calculating the mean proportion of MNs in buccal epithelial cells across all individuals and the percentage of micro-nucleated cells (%MN cells).

RESULTS

The study focused on mining workers aged 15–60 in Hospet and Sandur Taluk, with the duration of stay in the mining industry ranging from 1 to 35 years, and included four mines, with oral examinations and interviews for data collection.

Oral mucosal conditions

Out of 250 people employed in the mining area, 170 (68.0%) had no oral mucosal conditions, compared to 79 (32.6%) who did. Of them, 25 (10%) had leukoplakia, 1 (0.4%) had lichen planus, and 8 (3.2%) had ulcerations. One person (0.4%) had acute necrotising gingivitis, 2 (0.8%) had candidiasis, and 2 (0.8%) had abscesses. Thirty-nine people (15.6%) had OSMF, and two people (0.8%) had oral cancer [Table 1].

The study aimed to determine the number of MNs in oral mucosal cells of mining workers, comparing them to those without tobacco habits, and comparing them to those in non-mining areas as a control group for genotoxicity investigation.

In our investigation, MNs were found in all the study groups' exfoliated cells. Different study groups measured the presence of MN in exfoliated cells of the oral epithelium.

The following are the outcomes:

- Group I: Of the 30 individuals, 20 (66.7%) revealed an MN presence, whereas 10 (33.3%) did not.
- Group II: Of the 30 people in this group, 11 (or 36.7%) had MNs, whereas 19 (or 63.3%) did not.
- Group III: Of the 30 people, 5 (or 16.7%) had MNs, whereas 25 (or 83.3%) did not.

Statistical analysis of Chi-Square test showed the *P*-value of 0.000 is highly significant among the occurrence in different groups [Table 2 and Figure 1].

The frequency of MNs in exfoliated cells of the oral epithelium was calculated in each study group, and the results were contrasted with those of the control group.

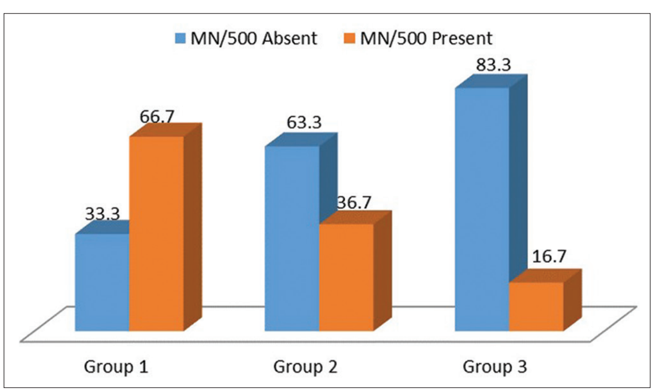

Figure 1: Occurrence of MNs in exfoliated cells of oral epithelium in different groups

In Group I, 10 (33.3%) individuals showed no presence of MN per 500 cells, 8 (26.7%) showed the presence of 1 MN per 500 cells, 5 (16.7%) showed the presence of 2 MNs per 500 cells, and 7 (23.3%) individuals showed the presence of minimum 3 to maximum 6 MNs per 500 cells. None of the individuals showed not more than 6 MNs per 500 cells.

In Group II, 19 (63.3%) individuals showed no presence of MN per 500 cells, 3 (10%) showed the presence of 1 MN per 500 cells, 6 (20%) showed the presence of 2 MNs per 500 cells, and 7 (6.7%) individuals showed the presence of minimum 3 to maximum 6 MNs per 500 cells. None of the individuals showed not more than 5 MNs per 500 cells.

In Group III, 25 (83.3%) individuals showed no presence of MN per 500 cells, 3 (10%) showed the presence of 1 MN per 500 cells, 2 (6.7%) showed the presence of 2 MNs per 500 cells, and none of the individuals residing in non-mining areas showed not more than 2 MNs per 500 cells.

Statistical analysis of Fisher's exact test showed the *P*-value of 0.000 is highly significant among the MNs of a number of frequency in different groups [Table 3 and Figure 2].

Comparing MNs per 500 cells amongst people in various groups was one of the study's goals. Out of 30 people working in the mining industry who had habits, we saw that 20 of them had MNs per 500 cells, ranging in number from

Table 1: Oral mucosal conditions

Condition	Frequency	Percentage
No abnormal condition	170	68.0%
Leukoplakia	25	10.0%
Lichen planus	1	0.4%
Ulceration (apthous, herpetic, and traumatic)	8	3.2%
Acute necrotising gingivitis	1	0.4%
Candidiasis	2	0.8%
Abscesss	2	0.8%
OSMF	39	15.6%
Oral Cancer	2	0.8%
Total	250	100.0%

Table 2: Occurrence of MN in exfoliated cells of oral epithelium in different groups

	Group			Total
	Group 1	Group 2	Group 3	
MN/500				
Absent	10 (33.3%)	19 (63.3%)	25 (83.3%)	54 (60.0%)
Present	20 (66.7%)	11 (36.7%)	5 (16.7%)	36 (40.0%)
Total	30 (100.0%)	30 (100.0%)	30 (100.0%)	90 (100.0%)

1 to 6 with a mean value of 2.40. Out of 30 individuals without habits, 11 individuals demonstrated the presence of MNs per 500 cells from minimum 1 to maximum 5 MNs per 500 cells, with the mean being 2.18; similarly, out of 30 individuals from non-mining areas, 5 individuals demonstrated the presence of MNs per 500 cells from minimum 1 to maximum 2 MNs per 500 cells, with the mean being 2.18 [Table 4].

DISCUSSION

An essential component of the mineral-rich Archaean Indian Shield is Karnataka. The state is well-endowed with a variety of precious metal ores, including those for iron, manganese, gold, copper, and chromium, as well as decorative rocks and beautiful minerals. Numerous environmental issues exist in and around the Sandur and Bellary region, according to the State Environment Report and Action Plan-2003 released by the Government of Karnataka. Due to issues brought on by mining, the area has been noted as one of the state's significant environmental hotspots. In addition to causing road damage, the movement of vehicles carrying iron ore is a major contributor to the region's extremely high levels of suspended particulate matter (SPM).[7]

Lead, cadmium, copper, zinc, and arsenic are the mining-related contaminants that have the highest toxicity levels for living things that are known to be carcinogenic and mutagenic.[8,9] Numerous research studies have examined the connection between elevated levels of heavy metals, their mutagenic and carcinogenic effects, and a rise in the incidence of malignant tumours in people.[10]

There are not many research studies on the impact of mining contaminants on dental health; therefore, it is impossible to determine for sure how serious of a concern these pollutants are for oral health. Determining the genotoxic impact of mining pollutants on oral mucosa of mine employees in Bellary region using the MN test is the goal of the current study, which is intended to evaluate the negative impacts of mining pollutants on oral soft tissues.

The Bellary district of Karnataka is where the current study was carried out. Specifically, Hospet and Sandur Taluk were chosen as the study's primary locations. In the study, four mines from the chosen Taluks were included. A survey was done, 250 mine employees between the ages of 15 and 60 were interviewed, and information was gathered. Before performing an oral examination, written consent was sought.

To determine the harmful impact of mining contaminants on the oral cavity, the oral cavity was studied in our study. Out of 250 people in the mining region, it was found that 170 (69.3%) were free of oral mucosal disorders, 25 (10%) had leukoplakia, 1 (0.4%) had lichen planus, and 8 (3.2%) had ulcerations. One individual (0.4%) had acute necrotising gingivitis, two (0.8%) had candidiasis, and two (0.8%) had abscesses. OSMF was detected in 39 (15.6%) people, and oral cancer was detected in 2 (0.8%). In an epidemiological investigation conducted in Malaysia by Demircigil *et al*. (1997), the prevalence of oral mucosal lesions was reported to be 9.2%, which is comparably lower than that of our research.[11]

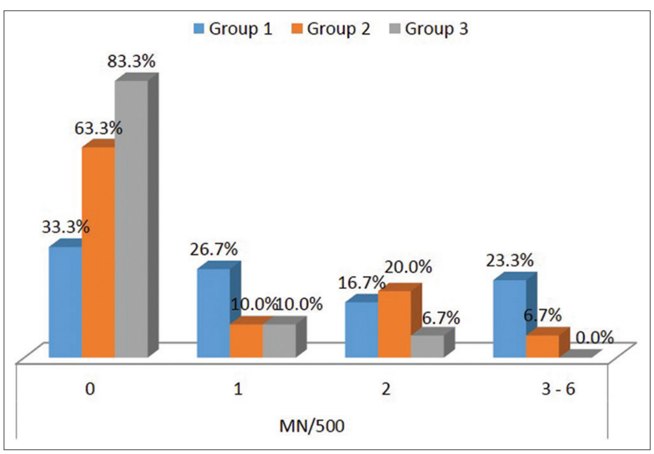

Figure 2: The number of MNs per 500 cells in different groups

Table 3: The number of MN per 500 cells in different groups

	Group			Total
	Group 1	Group 2	Group 3	
MN/500				
0	10 (33.3%)	19 (63.3%)	25 (83.3%)	54 (60.0%)
1	8 (26.7%)	3 (10.0%)	3 (10.0%)	14 (15.6%)
2	5 (16.7%)	6 (20.0%)	2 (6.7%)	13 (14.4%)
3-6	7 (23.3%)	2 (6.7%)	0 (0%)	9 (10.0%)
Total	30 (100%)	30 (100%)	30 (100%)	90 (100%)

Fishers exact test P=0.000, HS

Table 4: Frequency of MN per 500 cells in individuals of different group

		MN/500						
Group	*n*	Minimum	Maximum	Mean	Std. Deviation	Median	Kruskal Wallis test	*P*
Group 1	20	1.00	6.00	2.40	1.57	2.00	1.840	0.399
Group 2	11	1.00	5.00	2.18	1.25	2.00		NS
Group 3	5	1.00	2.00	1.40	0.55	1.00		
Total	36	1.00	6.00	2.19	1.39	2.00		

The MN test is a non-invasive, accurate screening method for early genotoxic damage in mine workers, detecting damage before cancer symptoms are apparent. However, a few studies have investigated the genotoxic potential of mining pollutants on workers. Our research group was split into two smaller groups, Group I, which consisted of people working in the mining industry with habits, and Group II, which consisted of people working in the mining industry without habits. These sub-groups were compared to a control group of 30 participants (group III) who had no exposure to mining contaminants.

Different groups were calculated for MN presence in exfoliated oral epithelial cells. Twenty of the 30 members of group I demonstrated the presence of MN, 11 of the 30 members of group II demonstrated the presence of MN, and 5 of the 30 members of group III demonstrated the presence of MN. In groups I, II, and III, there were 66.7%, 36.7%, and 16.7% MN per 500 cells, respectively. This suggests that group I and group II had more MNs per 500 cells than group III did.

The prevalence of MNs in exfoliated cells of the oral epithelium was calculated in several subjects among various groups, and the outcomes were compared with those of the control group. The Kruskal–Wallis test was used to compare MN frequency. In groups I and II, the mean MN was 2.40 and 2.18, respectively. The findings suggest that both groups can have genotoxic effects, which are shown as MNs.

When assessing MNs in the controls, five people had 1 to 2 MNs per 500 cells, with a mean of 1.40. This may be the case because MN development is not just a result of exposure to mining contaminants; it may also be a result of several other genotoxic agents, such as radiation, chemicals, or environmental pollutants. When compared to the control group, no statistically significant correlations were discovered.

When compared to the control group, the group of those who worked in the mining industry and had a history of tobacco-related addictions showed a 4-fold increase in MN frequency. When compared to the control group, the group of those who work in the mining industry and have no history of tobacco-related behaviours exhibited a 2-fold rise in MN frequency. This observation can be explained by the fact that clastogens, which break chromosomes, and aneugens, which damage the spindle machinery, both cause the creation of MNs.[12,13]

Our findings are consistent with a research by Duka DY *et al*. (2011), who found that children exposed to manganese mining had significantly higher mNUC levels than controls.[7] Although reported effects are minor, numerous studies show a significant increase in MN levels in exposed individuals.[3,14]

Research on the genotoxicity of mining contaminants in oral mucosa is limited. Increased MN-positive cells may indicate genetic harm and environmental mutagenesis effects.[15,16] The study suggests ongoing exposure could lead to harmful health risks. MN tests can be used as a preliminary indicator and buccal mucosal smears as a substitute.

CONCLUSION

The study in Karnataka, India, investigated the prevalence of tobacco and alcohol use, oral mucosal lesions, and the genotoxic effects of mining pollutants on mine employees. Results showed higher MN counts in mine employees than typical, healthy controls, suggesting that mining contaminants have genotoxic effects. The study suggests that prolonged exposure to mining may result in serious health risks and recommends using exfoliated buccal mucosal cells as a substitute of peripheral blood for identifying MNs in mining-induced genotoxic alterations.

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Nil.

Conflicts of interest
There are no conflicts of interest.

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