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Early detection of lung cancer potential among Egyptian wood workers

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ABSTRACT

Wood dust is known to be a human carcinogen, with a considerable risk of lung cancer. The increased cancer risk is likely induced through its genotoxic effects resulting from oxidative DNA damage. This study aimed at assessing the genotoxicity of wood dust and demonstrating the role of sputum PCR as a screening tool for early prediction of lung cancer among wood workers. The study was carried out in the carpentry section of a modernized factory involved with the manufacture of wooden furniture in Greater Cairo, Egypt. Environmental assessment of respirable wood dust concentrations was done. Frequency of chromosomal aberrations (CA%) and sister chromatid exchanges (SCE%) in peripheral blood lymphocytes (PBL) was assessed and comet assays were performed in samples from among the study population ($n = 86$). Levels of superoxide dismutase (SOD) and glutathione peroxidase (GPx) enzymes were measured. The polymerase chain reaction (PCR) was used to study hypermethylation of p16 and/or O^6-methylguanine-DNA methyltransferase (MGMT) gene promoters in sputum DNA. The concentrations of respirable wood dust exceeded the Egyptian and international permissible limits with highest levels generated by sawing operations. Laboratory investigations revealed statistically significantly higher frequencies of CA and SCE as well as increased comet tail length associated with significant decrement in the levels of SOD and GPx among exposed group. A statistically significant elevation in the extent of hypermethylation was detected for the p16 and MGMT gene promoters in the sputum DNA of studied wood workers. The study results support the conclusion that prolonged unprotected occupational exposure to wood dust is associated with possible genotoxicity and oxidative stress that might raise the risk for carcinogenesis including lung cancer.

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Introduction

The manufacture of wooden furniture in Egyptian carpentry factories, similar to wood work throughout the world, has been a major industry for several decades. Processing of logs generates wood dust that is a complex mixture of cellulose, polyoses, and lignin with a variable number of polar, non-polar, and water-soluble compounds [1].

Wood dust becomes a potential health problem when inhalable particles from different processes, as sanding, cutting, drilling, chipping, sawing, and turning to shape wood, become air borne. These particles might cause allergic and non-allergic respiratory symptoms, and skin and/or eye irritation in addition to the possibility of inducing varying cancers when deposited in the mucosa of nasal and paranasal sinuses, pharynx, and airways [2–4].

Consequently, numerous studies have assessed the relationship of wood dust exposure to the occurrence of cancers in the nasal and paranasal sinuses, lung, pharynx as well as the colon and rectum [2–7].

Genetic biomonitoring of populations exposed to potential carcinogens was commonly used as an early warning sign for genetic disorders and carcinogenesis. Accordingly, the genotoxicity of occupational exposure to wood dust may be detected in the peripheral blood lymphocytes (PBL) using comet assay and analysis for chromosomal aberrations (CA) and sister chromatid exchanges (SCE) [1].

Also, biochemical estimation of radical scavenging antioxidant enzymes, like superoxide dismutase (SOD) and glutathione peroxidase (GPx), gives information on the possible oxidative stress responsible for the developing DNA damage and carcinogenic mechanism [8].

Fortunately, lung carcinoma is a key example of a cancer for which mortality could be greatly reduced through development of sensitive molecular markers detectable at the earliest stages of the disease [9].

The recent increase of polymerase chain reaction (PCR) sensitivity in studying methylated DNA sequences has led to detection of p16 and/or O^6-methylguanine-DNA methyltransferase (MGMT) gene promoters' hypermethylation in the DNA from sputum in 100% of

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patients with lung carcinoma up to 3 years before clinical diagnosis. Thus, the use of gene hypermethylation as a molecular marker system seems to offer a potentially simple and non-invasive screening tool for at risk populations, such as workers exposed to wood dust with higher incidence for lung cancer [9,10].

Methods and materials

Study design and population

This paper reports the results of a cross sectional study performed in the carpentry section of wooden furniture manufacturer in an Egyptian Modernized Industrial factory located in Greater Cairo.

The studied population comprised a total of 86 subjects divided into 43 exposed and 43 control individuals. The exposed group included those male workers from the carpentry section who have been exposed to wood dust at the workplace (mixture of soft and hard wood) for more than 5 years. Thus, a total of 43 workers with duration of exposure extending from 6 to 31 years were included in the study. The study excluded 42 wood workers before starting the study based on the pre-determined exclusion criteria (i.e. the total number of the workers in this carpentry section was 85).

Exclusion criteria:

Exposures with other chemical exposures at workplace in addition to wood dust. (NB: Combined exposure to chemicals like solvents or adhesives, for example, was excluded [by history taking and workplace inspection] to avoid their effect on the results of genotoxicity and oxidative stress assessment. This could ensure that the detected results were attributed to wood dust exposure only).

Present history of hypertension or diabetes.

Familial history of any type of cancer.

Moderate to heavy smokers (Smoking Index ≥ 20 pack. years).

Abnormal liver function tests.

The control group was selected from the administrative department of Kasr Al-Ainy hospital to include 43 matching subjects with no history of previous exposures to wood dust. The subjects were selected with comparable age, sex, special habits of medical importance, and socioeconomic standards to the exposed group. The previously mentioned exclusion criteria (apart from the first one) were also applied to the control group.

The study was approved by the Ethical Committee of the Occupational and Environmental Medicine Department, Kasr Al-Ainy Hospital, Faculty of Medicine, Cairo University. Approval was also taken from the Chief Executive Officer (CEO) of the factory. Written informed consent for sharing in the study was voluntarily obtained from each individual after proper explanation of the aim and objectives of the current study. During the study, the ethical guidelines of good clinical practices (GCPs) and strict confidentiality were ensured throughout sample collection, coding, testing, and recording of results.

Methodology

A. Environmental assessment

During an average workday, measurement of respirable wood dust levels in air was carried out at five different sites in the carpentry section of the Egyptian modernized factory. The sites included the areas for band saw, ripsaw, orbital sander, shaper, and thickness planer. Local exhaust ventilation (LEV) was installed in the area, but was not sufficient to clear the workplace air from formed dust to the extent that the air suspended dust was clearly observable without instruments. Also there was no obvious interest among workers to use the provided respiratory protective equipment (RPE) as most workers claimed feeling discomfort with its use mainly due to improper sizes and interference with any precise process in the work.

A total number of eight air samples were collected at each working site using the Haz dust respiratory particulate monitor. The readings were recorded at each machine site every one hour for eight successive hours to represent the whole sampling period of the entire work shift. Samples were also collected at the workers' breathing zone using the personal sampling pumps. Each individual personal pump was left for 8 h during the whole work shift to obtain a close reading representative of the actual full-shift exposure. For the personal sampling, the dust was collected on pre-weighed Whatman GF A (glass fiber) filters. It was mounted on an open filter holder. The air was aspirated by the personal vacuum pump (air flow was 2L/min). After sampling, the filters were dried over silica gel for at least 24 h before being re-weighed.

According to Wright's method [11], the respirable dust (<5 μm) concentration was measured and expressed in mg/m^3. The mean and standard deviations were calculated for the 8 hourly readings at each machine location.

B. Clinical assessment

Medical history taking. The study population was asked about the personal history including age, residence, socioeconomic standards, smoking, and other special habits of medical importance. Full occupational history was recorded from all participants concerning the type and duration of the current job, exposure hours, usage of respirators, goggles, gloves, suits/aprons, or special shoes/boots. The participants were also asked about if they were involved in any associated jobs currently as well as the presence of any previous occupations or exposures to hazardous materials. Whether such exposures were the reasons behind changing work was also stressed.

The present history concentrated on any physiological systems affected with emphasis on respiratory

manifestations in addition to eye irritation or skin lesions. The potential participants with diabetes mellitus and hypertension were excluded. Past history was checked for any chest diseases (*tuberculosis*), or liver disease (*bilharziasis or hepatitis*), intake of regular medications, occupational injuries and sick leaves, and previous operations or past history of malignancy. Family history of bronchial asthma, skin allergies, or malignancies was also considered.

Clinical examination. Thorough general examination comprised measuring all vital signs as pulse, blood pressure, respiratory rate, and temperature. The eyes were externally examined for redness and tearing. The skin was inspected for redness, dryness, cracks, itching marks, and blisters. Full local chest and abdominal examinations were performed to exclude any coexisting diseases.

C. Laboratory investigations

Comet assay. Small samples (40 mcl) were taken from collected blood and the assay was carried out according to published methods [12] with slight modifications [13]. Cell viability was determined by the trypanblue exclusion technique and ranged from 94 to 96%. All coded slides were scored and a total of 100 individual cells were screened per individual case taking into consideration the inter-scorer variability. The length of DNA migrating in the comet tail was measured by an ocular meter.

CA and SCE study. Analysis for the presence of CA was carried out using the modified standard protocol [14]. Prepared slides were analyzed and 200 metaphase cells for each case were screened for chromatid and chromosomal breaks, chromatid deletions, chromatid rings, dicentrics, and acentric fragments.

Antioxidant enzyme assays. Samples from collected blood were subjected to protein determination [1]. Both SOD and GPx activities in serum were measured by spectrophotometrically [15,16].

PCR for sputum DNA. Sputum was collected in a plastic cup from each individual case early in the morning after provision of required instruction. Sputum samples were preserved in Saccomanno's fluid until subjected to cytological examination to confirm the presence of bronchial epithelial cells [17]. Genomic DNA was then extracted and analyzed to detect hypermethylation of gene promoters for p16 and MGMT [18,19].

Table 1. Means and Standard Deviations (SD) of respirable wood dust levels at 5 different locations.

	TWA Samples	
Locations	Personal sample	Haz dust monitor (n=8) Mean ± SD
Band saw	12.57	13.27 ± 2.03
Ripsaw machine	10.85	11.06 ± 0.56
Orbital sander	8.64	9.34 ± 1.23
Shaper	6.92	7.16 ± 1.69
Thickness planer	5.73	6.89 ± 0.91

TWA: Time weighted average (mg/m^3).

D. Statistical analysis

Data obtained from the study were coded and entered using the statistical package for social sciences (SPSS-22). Data were analyzed using the mean and standard deviation for quantitative variables and the frequency (count) and relative frequency (percentage) for categorical data.

The comparisons between data related to exposed and control groups were done using the Chi square (Chi^2) test for qualitative variables while the Fisher exact test was used with expected frequency less than five. The independent simple t-test was used for normally distributed quantitative variables. One way ANOVA was used for multiple comparisons. For CA and SCE assays, the percentages represented the mean frequencies of the specified endpoints in the exposed workers and control subjects.

Odds ratio (OR) with 95% confidence intervals were calculated using binary logistic regression. *P*-values less than 0.05 and less than 0.001 were considered statistically significant and highly significant, respectively.

Results

The relative accuracy of the environmental assessment could be evidenced by the observed close alignment of personal exposure values with the environmental monitoring results. The values of environmental assessment at different locations in the carpentry section revealed the presence of highest concentrations of respirable wood dust at the Sawing process (Table 1). The workers were exposed to soft and hard wood particles for employment (exposure) duration of 14.72 ± 5.41 years (range, 6–31 years).

The carpenters included in the study were 43 male workers of age range from 27 to 58 years with a mean value of 40.79 ± 8.6 years showing no statistically significant difference when compared with the control group (mean, 41.37 ± 8.18 and range, 28–57 years). The smoking index was low among the included study population with no statistically significant difference between both exposed and control groups being 11.81 ± 4.02 and 10.98 ± 6.08 pack.years, respectively.

Clinically, both upper and lower respiratory manifestations as well as contact dermatitis and eye irritation were increased among exposed workers compared to the non-exposed group with a highly significant statistical difference. It is worthy to mention that all exposed workers (n = 43, 100%) were complaining of respiratory manifestations mainly in the form of nasal hypersecretion, nasal blockage, dyspnea, cough, expectoration, sneezing, and wheezy breathing. Skin manifestation in the form

of contact dermatitis was observed among around 33 (76.7%) exposed workers. The affected regions were mainly the face, hands, forearms, and neck. Eye effects, in the form of burning sensation, irritation, and redness, were detected among 30 (69.7%) workers of the exposed group. Concerning the control group, the frequencies of respiratory, skin, and eye manifestations were 18.6, 4.7, and 9.3%, respectively.

The results of comet assay as well as the frequency of CA and SCE among the study groups showed high significant elevations ($p < 0.001$) among workers exposed to wood dust (11.89 ± 2.42 µm, 7.51 ± 1.24%, 7.66 ± 1.09%, respectively) compared to the non-exposed (6.60 ± 1.25 µm, 3.57 ± 0.79%, 3.46 ± 0.78%, respectively). Concerning the subtypes of chromosomal aberrations, results showed a highly statistically significant increase among the exposed group compared to controls ($p < 0.001$). The observed aberrations included chromatid breaks (2.73 ± 1.28%), chromosome breaks (2.59 ± 1.14%), deletions (2.36 ± 0.65%), dicentrics (2.04 ± 1.01%), acentric fragments (1.35 ± 0.60%), and finally ring chromatids (1.25 ± 0.20%) compared to 1.13 ± 0.30, 1.07 ± 0.45, 0.93 ± 0.35, 0.78 ± 0.12, 0.40 ± 0.11, and 0.35 ± 0.12%, respectively, among the control group. These figures represented the percentage of subjects with the specified endpoints.

In Table 2, a highly significant lowering in the serum level of SOD and GPx antioxidant enzymes was detected among the exposed group versus the control.

Also, the current study revealed a statistically significant difference between the exposed and control groups as regards the methylation of p16 gene promoter (OR 11.118, 95% CI 1.341–92.150). A highly statistically significant difference was observed between the two studied groups for the methylation of MGMT gene promoter. This increased state of methylation of both genes and suggests an increased lung cancer risk based on gene hypermethylation (OR 22, 95% CI 2.965–163.213) where the hypermethylation of at least one gene only of both studied genes could represent a significant risk for lung cancer incidence [9] (Table 3). Due to the wide confidence limits, the confidence in the actual value of the OR is low, although there is a clear difference between the groups.

The exposed group was further subdivided according to the state of both genes' methylation. No statistically significant difference was revealed between the subgroups based on age or smoking index. Statistically significant higher values were obtained for the number of years related to wood exposure (employment), length of comet tail, and frequency of CA and SCE. Also, statistically significant decreases in the levels of antioxidant enzymes (SOD and GPx) were shown among the methylated subgroup (Table 4). A Post hoc test revealed a statistically significant increase in the length of comet tail among wood workers with methylation of both genes. Carpenters with methylation of the p16 gene only had a significantly increased number of CA with decreased SOD level. On the other hand, wood workers with methylation of the MGMT gene only had a significant increase in the number of SCE with decreased GPx level. Also the statistically significant difference between subjects with both genes methylated and those without could be attributed to the prolonged unprotected duration of exposure (Table 4).

Correlation and liner regression analysis for the duration of exposure versus the different laboratory parameters revealed a positive association with the length of comet tail ($r = 0.742, \beta = 6.275$) as well as the frequency of CA ($r = 0.614, \beta = 5.085$) and SCE ($r = 0.702, \beta = 6.095$). A negative association was detected with both antioxidant enzymes; SOD ($r = -0.619, \beta = -4.040$) and GPx ($r = -0.561, \beta = -4.834$). All associations were highly significant statistically (p value < 0.001).

Table 2. Means and SD of comet tail length, CA%, SCE%, SOD, and GPx among both exposed and control groups.

	Exposed (43)	Control (43)	
Investigation	Mean ± SD	Mean ± SD	t-test
Comet (µm)	11.89 ± 2.42	6.60 ± 1.25	12.714*
Total CA %	7.51 ± 1.24	3.57 ± 0.79	17.584*
SCE %	7.66 ± 1.09	3.46 ± 0.78	20.527*
SOD (U/mg)	0.73 ± 0.33	2.36 ± 0.65	−13.716*
GPx (U/mg)	31.44 ± 9.50	56.15 ± 7.57	−13.334*

*Highly significant t-test, p value < 0.001.

Table 3. Comparison between the group of workers exposed to wood dust and the control group as regards the sputum PCR results.

	Exposed (n=43) control (n=43)	
Gene promoters	No. % No. %	Chi^2 Odds ratio 95% CI lower upper
p16	Unmethylated 34 79.1 42 97.7	7.242† 11.118 1.341 92.150
	Methylated 9 20.9 1 2.3	
MGMT	Unmethylated 29 67.4 43 100	16.722*
	Methylated 14 32.6 0 0	
Both genes	Unmethylated 22 51.2 42 97.7	16.722*
	One only methylated 19 44.2 1 2.3	
	Methylated 2 4.7 0 0	
Net result	Unmethylated 22 51.2 42 97.7	26.174* 22 2.965 163.213
	Methylated 21 48.8 1 2.3	

MGMT, Methylguanine DNA methyl-transferase.
†Significant Chi^2 test, p value < 0.05.
*Highly significant Chi^2 test, p value < 0.001.; NB: The uncalculated odds ratios are due to the presence of frequency zero of methylated category in the control group.

Discussion

Respirable wood dust particles with aerodynamic diameter less than 5 µm can deposit in the lower airways with the increased possibility of causing significant health hazards [20]. The environmental assessment of wood

Table 4. Means and SD of age, SI and duration of exposure, comet tail length, CA%, SCE%, SOD, and GPx levels among the subgroups of exposed population ($n = 43$).

	Methylation (21)					
	P16 (n=7)	MGMT (n=12)	Both (n=2)	Net (n=21)	Non-methylation (22)	ANOVA
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD		
Age (years)	38.43 ± 8.30	43.33 ± 7.93	42.5 ± 18.92	41.05 ± 9.38	39.67 ± 7.86	0.521
SI (Pack.years)	8.14 ± 2.47	12.5 ±3.28	12 ±4.04	11.64 ± 3.06	10.95 ± 3.00	0.916
Exposure (years)	18.71 ± 4.92	13.5 ±3.55	30 ±1.41†	16.59 ± 6.22	11.76 ± 6.15†	2.029*
Comet (µm)	11.01 ± 1.82	12.82 ± 1.66	13.16 ± 1.35†	12.98 ± 1.65	9.74 ± 2.60†	3.363*
Total CA %	7.85 ± 1.23†	7.88 ± 0.62	9.22 ± 0.01	8.97 ± 0.90	7.11 ± 0.68†	3.714*
SCE %	8.28 ± 0.82	7.93 ± 0.69†	8.61 ± 1.19	8.87 ± 0.77	7.13 ± 0.75†	3.626*
SOD (U/mg)	0.67 ± 0.11†	0.52 ± 0.27	0.53 ± 0.03	0.58 ± 0.25	0.89 ± 0.43†	−2.502*
GPx (U/mg)	28 ± 10.14	30.22 ± 8.60†	19.50 ± 1.56	26.55 ± 8.96	34.47 ± 9.29†	−2.126*

SI, Smoking Index; and MGMT, Methylguanine DNA Methyl transferase.
*Significant ANOVA test, p value < 0.05.; †Significant Post hoc test in relation to genes methylation, p value < 0.05.

dust over the shift work duration (8 h TWA) at five different locations in the carpentry section exceeded Egyptian Environmental Law No.4 that set a TLV-TWA of 5 mg/m^3 for hard and soft wood dust [21]. Internationally, recommended exposure limit (REL) for all hard and soft wood dust is even lower, TWA of 1 mg/m^3 [22]. Recently a TLV-TWA of 1 mg/m^3 for hard wood dust, and a TLV-TWA of 5 mg/m^3 for soft wood dust were set [23].

Environmental monitoring of wood dust exposure at the workplace could be achieved by measuring total dust, inhalable dust, and respirable dust. However, the most important type of dust from the clinical point of view is the respirable dust with particles less than 5 µm in diameter, thus easily deposited in the lower airways and causing significant health hazards [20]. The highest levels of respirable dust were generated during the sawing process by either the band saw or ripsaw posing a greater risk for exposed workers who are not wearing any RPE. Egyptian primitive carpentry workshops have always been suggested as potential critical source for wood dust generation [24].

A similar earlier study has discovered that indoor sawing of wood sheets with poor ventilation could yield very high levels of wood dust up to 25.8–34.9 mg /m^3 in just 2 h of continuous work [25]. Therefore, the control of wood dust hazardous to health requires regular assessment and proper management to prevent extensive exposure at the source. The best way is by ensuring the presence of effective LEV. The use of RPE is not a substitute but only considered as a temporary measure for additional protection [26]. However, previous studies recommended the use of RPE as its lack could adversely influenced woodworkers' respiratory functions [27,28]. This is due to enhanced exposure to wood dust, and possibly an increased risk for lung cancer.

Almost all workers in the carpentry section were subjected to continuous variable levels of wood dust exposure. There was no strict job specification as workers used to shift from one machine to another in the factory. Besides, all machines were found in a single large area with only few meters (3–5 m) distance between machines. This workspace layout posed some difficulty in assessing the dose response relationship for workers from specific operations. This is different from very early studies reporting clear dependence of cancer risk on the job specification of exposed groups [29].

The exposed wood workers from the carpentry section presented with many upper and lower respiratory manifestations ($n = 43$, 100%) besides contact dermatitis ($n = 33$, 76.7%) and eye irritation ($n = 30$, 69.7%). Even at a mean exposure of around 1 mg/m^3, wood dust has been regarded as an irritant to the respiratory mucous membranes [30].

Occupational contact dermatitis was reported as dose-dependent finding among workers exposed to wood dust [31]. The presence of burning sensation and irritation or redness of the eyes was also documented among carpenters [32].

In the current study, there was no clear history of malignancy among exposed workers with emphasis on lung cancer. Unfortunately, there was no available accurate documentation in the factory to know the overall cancer rate among workers and even if there was a history of a former cancer cases or cancer mortality, there was no determination of the type of cancer.

Genotoxicity among wood workers was evidenced by increased frequency of CA and SCE in PBL (7.51 ± 1.24 and 7.66 ± 1.09%, respectively) compared to the non-exposed group (3.57 ± 0.79 and 3.46 ± 0.78%, respectively). Earlier studies reported statistically significant higher frequencies of CA% and SCE% among carpenters involved in wooden furniture industry. The abnormalities reported included chromatid breaks (4.41 ± 0.76), chromosome breaks (3.71 ± 0.58), dicentrics (2.76 ± 0.49), acentric fragments (1.86 ± 0.67), and chromatid rings (3.20 ± 0.63) [1]. Even higher values were detected among workers in small furniture workshops in Cairo with chromatid breaks (5.53 ± 3.68), chromosome breaks (7.10 ± 3.34), deletions (5.28 ± 2.20), dicentrics (4.64 ± 1.76), and finally ring chromatids (1.21 ± 1.20) [22].

Additionally, increased length of comet tail was detected in PBL of wood workers with a highly significant difference with the non-exposed group. Comet assay is considered a very sensitive and rapid technique to quantify and analyze any DNA damage in the

individual cells. Increased comet tail length is an indication for increased number of DNA breaks as the DNA loops showing breaks lose their supercoiling properties and become free movable [33].

The DNA-damaging effect of wood dust was attributed to the production of reactive oxygen species with the release of tumor necrosis factor-alpha and macrophage inflammatory protein-2 in alveolar macrophages exposed to these particles. Peroxidation of lipids, disruption of DNA and proteins, exertion of signaling functions, and modulation of gene transcription are all the results of reactive oxygen species [34].

Superoxide dismutase is an enzyme that catalyzes the conversion of the superoxide (O_2^-) radical into hydrogen peroxide (H_2O_2), which is further reduced to water by glutathione peroxidase enzyme. Both enzymes play a critical role in the inhibition of oxidative stress with an attempt to protect the oxygen-metabolizing cells against the harmful effects of reactive oxygen species [35].

Analysis of antioxidant enzymes in the serum of wood workers revealed marked reduction indicating the excess consumption of these enzymes during the critical state of oxidative stress. The levels of SOD and GPx were significantly lower among the exposed group, being 0.73 ± 0.43 U/mg protein for SOD and 31.44 ± 9.50 U/mg protein for GPx compared to 2.36 ± 0.65 U/mg protein and 56.15 ± 7.57 U/mg protein among control group, respectively.

Concerning the early prediction of lung cancer incidence, a reliable non-invasive screening tool which is a specific PCR approach was used to detect the hypermethylation of a wide panel of gene promoters in the sputum DNA. The hypermethylation of at least one gene is sufficient to predict lung cancer among high risk exposed group [9,36].

Highest specificity was seen for p16 gene [37], as this tumor suppressor gene is inactivated at prevalence of up to 67% in adenocarcinoma and 70% in squamous-cell carcinoma of the lung [38,39]. Taken together, both p16 and MGMT genes have been considered potentially useful candidate biomarkers for early detection and prediction of lung cancer by improving the sensitivity and specificity of this screening approach [36,40]. The promoter hypermethylation of both genes is an early and very frequent event in primary lung tumor cells and in exfoliated cells in the sputum of cancer patients and in high risk cancer-free individuals [9].

Gene silencing or inactivation through methylation of cytosine adjacent to guanosine in C-phosphate-G (CpG) islands, in conjunction with chromatin remodeling lead to the development of heterochromatin of the gene promoter region, which denies access to regulatory proteins needed for transcription. This epigenetically driven process is a major and causal event silencing hundreds of genes involved in all aspects of normal cellular function during lung cancer initiation and progression resulting in loss of tumor suppressor gene function among cancer cases [41]. Importantly, silencing of genes, such as cyclin-dependent kinase inhibitor 2A (CDKN2A or p16) and /or MGMT genes after being hypermethylated in sputum, was associated with a very high sensitivity and specificity in early prediction of lung cancer [36,42].

The results of current study revealed a statistically significant difference in the sputum PCR analysis for both p16 and MGMT gene promoters for workers occupationally exposed to wood dust compared to the control group. This finding is evidenced by the higher frequency of methylation for p16 (20.9%) and MGMT (32.6%) gene promoters and hence potential increased risk for lung cancer among wood dust exposed workers. A great association was demonstrated between exposure to wood dust at work and presence of gene promoter hypermethylation as evidenced by the values of odds ratio being 11 for p16 gene and 22 for the net result of lung cancer risk evidenced by gene methylation with 95% confidence intervals 1.341–92.150 and 2.965–163.213, respectively (Table 3).

The increased incidence of lung cancer was previously found to be associated with wood dust exposure in a nested case control study among workers exposed to high concentrations of wood dust in a pulp and paper mill [43]. Similarly, an increased risk of lung cancer was reported among carpenters exposed to wood dust for more than 30 years [44]. Another earlier study observed an excess of lung cancer cases associated with many wood dust-related employments, including forestry occupations, processing of wood and wood products, wood machining, fabrication, and repair of wood products in addition to carpentry [2].

Recently, the evidence of increased risk of lung cancer among workers with substantial cumulative exposure to wood dust was confirmed. Two population-based case control studies involving wood dust exposed subjects in construction, timber, and furniture making industries reported increased lung cancer risk in Study I (*OR* = 1.4, 95% confidence interval 1.0–2.0) and in Study II (*OR* = 1.7, 95% confidence interval 1.1–2.7) [45].

The exposed wood workers with gene methylation showed marked consumption and lowered antioxidant enzymes level of statistically significant difference with unmethylated workers. This decrease was significantly associated with increased frequency of chromosomal aberrations, sister chromatid exchange and length of comet tail (Table 4). Likewise, other studies confirmed the existence of a strong relationship between both p16 and MGMT gene promoters' hypermethylation and unrepaired DNA damage detected by comet assay [46].

No statistically significant difference between the methylated and unmethylated individuals of the exposed group in age and smoking index as previously suggested in similar studies [47,48]. However, a significant increase in gene promoters' methylation was recognized for the

duration of employment thus providing a reflection on the fact that gene methylation status is greatly affected by the total number of years of exposure to the deleterious agent and hence elevated risk for lung cancer. Correlation and linear regression analysis indicated that the duration of work exposure can be considered a predicting factor for high frequency of chromosomal changes and sister chromatid exchange as well as the presence of unrepaired DNA damage evidenced by increased comet tail length. This finding was emphasized by a recent study confirming the presence of increased risk of lung cancer among workers in relation to the substantial cumulative exposure to wood dust for 5 years or more [45].

Conclusion

The current study might direct the concern towards the potential hazards of wood dust exposure. The existence of chromosomal and DNA affection supports the conclusion that wood produces toxic dust capable of influencing the occupationally exposed population and predisposing the workers to lung cancer. Consequently, the use of sputum PCR for detection of aberrant gene methylation seems to offer potentially simple and non-invasive screening tool among woodworkers to identify those with higher risk for lung cancer.

Importantly, great attention should be directed to control measures that protect multiple at-risk workers at a time. Personal protective equipment should not be the only control measure to be considered. Elimination and engineering controls including the local exhaust ventilation in the workplace are more effective and should be subjected to strict maintenance programs.

Disclosure statement

No potential conflict of interest was reported by the authors.

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