

Evaluation of DNA Damage for Some Iraqi Workers Employ in a Wooden Furniture Factories

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Abstract

Wood is among the most universe substantial resources and one of the main common kinds of occupational exposure. For this , the research goal was the assess of the genotoxicity effects of wood dust exposure for some Iraqi workers employed in a wooden furniture factories using alkaline comet assay based on measuring the DNA damage that occurred in the white blood cells (WBC) , aimed to minimize the health risks from dangerous substances in the workplace. Fifty workers in a wooden furniture factories and 50 apparently healthy control were used in this study. DNA damage was significantly higher in the wood's workers , than in the control subjects according to the comet parameters. Thus , significantly higher levels of DNA damage observed in wood's workers in whom either smoked (tail length 40.15 ± 0.54 ; tail DNA % 32.12 ± 0.87 and tail moment 12.90 ± 0.98) or non-smokers (tail length 36.21 ± 0.43 ; tail DNA % 29.56 ± 0.34 and tail moment 10.70 ± 0.10) than in smoker (tail length 12.81 ± 0.89 ; tail DNA % 6.60 ± 0.06 and tail moment 0.846 ± 0.02) and non-smokers (tail length 2.63 ± 0.33 ; tail DNA % 2.29 ± 0.07 and tail moment 0.060 ± 0.03) of control group. Present study deduce the relationship of the exposure to wood dust with high level of DNA damage. In conclusion, the results indicated that there was a possibility of using the changes in the level of comet assay as try for the detection of DNA damage of workers employed in a wooden furniture factories . Also, the results obtaining was confirmed by usefulness of the alkaline comet assay as a sensitive additional marker in the regular health screening of workers occupationally exposed to dangerous wooden dust .

Key words: *Wood Dust; Genotoxicity ; DNA Damage ; Comet Assay .*

تقييم تلف الحمض النووي منقوص الاوكسجين لبعض العمال العراقيين العاملين في مصانع الأثاث الخشبية

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المستخلص

يعتبر الخشب من معظم الموارد الكبيرة و أحد الأنواع الرئيسية الشائعة للتعرض المهني المعروف. لهذا، كان الهدف من هذا البحث تقييم التأثيرات السمية الوراثية لنشارة الخشب على بعض العمال العراقيين العاملين في مصانع الأثاث الخشبي، اعتمادا على قياس تلف الحمض النووي منقوص الاوكسجين الذي يحدث في خلايا

الدم البيضاء (WBC) من اجل الحد من المخاطر الصحية الناجمة عن المواد الخطرة في مكان العمل. خضع لهذه الدراسة خمسون عاملا في مصانع الأثاث الخشبي باستخدام تقنية المذنب القلوية و 50 شخص اصحاء. كان تلف الحمض النووي منقوص الاوكسجين أعلى وبدرجه معنويه في عمال مصانع الأثاث الخشبي ، مما كان عليه في المجموعة الضابطة وفقا لمعايير المذنب. حيث لوحظ ارتفاع معنوي في مستويات تلف الحمض النووي منقوص الاوكسجين لدى عمال مصانع الأثاث الخشبي المدخنين منهم (طول الذيل 0.54 ± 40.15 ، النسبه المئوية للحمض النووي في الذيل 0.87 ± 32.12 % و لحظة الذيل 0.98 ± 12.90) أو غير المدخنين (طول الذيل 0.43 ± 36.21 ، النسبه المئوية للحمض النووي في الذيل 0.34 ± 29.56 % ولحظة ذيل 0.10 ± 10.70) مما كان عليه في المدخنين (طول الذيل 0.89 ± 12.81 ، النسبه المئوية للحمض النووي في الذيل 0.06 ± 6.60 % و لحظة الذيل 0.02 ± 0.846) وغير المدخنين (طول الذيل 2.63 ± 0.33 ، النسبه المئوية للحمض النووي في الذيل 0.07 ± 2.29 % ولحظة الذيل 0.03 ± 0.060) من المجموعة الضابطة. دلت ادراسته على ان هناك علاقة بين التعرض لغبار الخشب والمستوى المرتفع لتلف الحمض النووي. يستنتج من ذلك، ان النتائج تشير إلى أن هناك إمكانية استخدام التغييرات في مستوى مقايسة المذنب كمنتج بايولوجي للكشف عن تلف الحمض النووي منقوص الاوكسجين لدى عمال مصانع الأثاث الخشبي . كما اكدت النتائج على ان فحص المذنب القلوي يعد معلم بايولوجي حساس إضافي يستخدم في المسح الصحي المنتظم للعمال المعرضين مهنيا لخطورة مواد نشارة الخشب.

الكلمات المفتاحية: غبار الخشب، السمية الوراثية ، تلف الحمض النووي منقوص الاوكسجين، فحص المذنب.

Introduction

Some of the dusty tasks such as sawing, drilling and furniture are manually done. A part of the sawdust always escapes the filtration systems of the machines causing an exposure of the workers at the facility [25] . According to International Agency for Research on Cancer (IARC), the wood dust was rating as a human carcinogenic (group 1), for its high carcinogenic ability to induce sinonasal cancer [9 ; 10] .

A large and diverse number of health effects are correlated with the exposure to the wood dust , non-cancerous ones implicate agitation and inflammation of the respiratory tract which give rise to coughing, wheezing, chronic bronchitis, and asthma [11]. The adverse effects of microorganisms are manifested by alveolitis allergica and organic dust toxic syndrome (ODTS), also it can induce aspergillomycosis , bronchial asthma, rhinitis and allergic dermatitis [23] . The irritant and allergic effects of wood dust have been recognised for a long time. Some of the allergic effect was caused by the wood dust [28] . Wood dust particles cause mucosal , allergic and non-allergic respiratory symptoms and cancer when they get deposited in nose, throat and other airways [3,18 and 26] . It has been suggested that exposed to wood dust will be associated with genotoxicity and related to extremely elevating risk of cancer [6,10 ,11 , and 22] . Many types of cancers , such as cancer of lung , pharynx and stomach and adenocarcinoma of nasal cavity and Hodgkin's disease , colorectal cancer has been

confirmed from several studies on human populations exposed to wood dust [5, 16 and 27] .

Genetic follow-up for people exposed to carcinogens is a system of early detection of genetic diseases and cancer as it can focus on the risk factors at right time where the opportunity is still available for the implementation of control [14] . The comet assay was deemed as an appropriate for genetic biomonitoring studies and ascertain occupational exposure to wood dust [20 , 22] .

As yet, in Iraq there is finite data available on the genotoxicity of genetic alterations which caused by exposure to wood dust. Subsequently , the present study's goal , was at the estimation of the genotoxicity relevelated to wood dust exposure. As, the comet assay has become an substantial tool in the area of human genotoxicity bio-monitoring studies and it was widely used in human genotoxicity monitoring in Iraq [2 , 4] . The single cell gel electrophoresis assay in individual cells was adapted in this study for detecting the level of DNA damaged in WBC of some Iraqi workers employed in a wooden furniture factories.

Materials and Methods

Subject

The study was conducted on 50 male who were working in different carpentry units in Baghdad, Iraq. They were occupationally exposed to the soft and hard wood dust , all the informations from the carpenters in the present study reveales that both hard wood and soft wood dust exposure were known to be routinely using in combinations through furniture industry. While , the control group included 50 apparently healthy men with no history of exposure to wood dust .

The subjects criteria were based on a questionnaire. Its included standard demographic data (age) , medical (guaranteed that all subjects had not been taking any medications or vaccinations , nor exposed to any type of radiation for one year before sample collection) , lifestyle (each subject smoked more than five cigarettes per day and each subject took five glasses or more of an alcoholic drink per day for 1 year before sample collection , was considered as smokers and alcohol drinkers , respectively) and vocational questions (working hours/day, years of exposure , precautionary measures taken , etc.) . The study selected on the subjects who had worked for 5 years or more in the carpentry field . The agreement of each subject was obtained to undergo his blood sample for this study after informing him about the goal of the research.

Blood sampling

Blood samples were collected from all the exposed worker and healthy individuals . Sampling was carried out over a period of 5 months , the samples were coded to avoid the possibility of bias. A 3 ml of venous blood was collected once from all the exposed and control subjects using heparinized syringes. The samples were transported immediatly on ice to the laboratory [12] .

Comet assay

DNA damage analysis for all subject in this study (exposed and control) was done using the comet assay according to [24] . Comet slides were prepared in duplicate

per subject . Ten microliter of blood samples were mixed with 75 μ l of 0.5% low melting point agarose . The mixture was cast into previously coated frosted slide with 0.5% normal melting point agarose and allowed to gel to be solidified. Then it were placed in cold lysis buffer (2.5 M NaCl , 10 mM Tris-base , 100 mM disodium-EDTA, 1% Na-sarcocinat, 10% DMSO, 1% Triton X-100, pH 8) for 2 hour at 4°C before DNA was treated with alkaline solution (0.3 M mNaOH, 1 mM EDTA-NA2, pH 13.2) for 20 minutes to allow for unwinding of the DNA strands. Following , alkaline electrophoresis was run for 30 min at 300 mA and 24 volts , the DNA strands migrate toward the anode according to size. The extent of migration depends on the number of strand breaks in the nucleoid. The electrophoresis slides were neutralized by washing twice for 5 min in neutralizing buffer (0.4 M Tris, pH 7.5) and once in water before dehydration in 100% ethanol for 20 min, and then it dried in 50°C for 30 min to be stored until use. For examination , stored slides rehydrated with chilled water for 30 min and it stained with 85 μ l Ethidium bromide for 5 min and the slides washed to remove the excess of stain , covered with coverslip. The migration of damaged DNA was visualized under a fluorescence microscope at 400x magnification, with a 450-490 nm emission filter and 515 excitation filter . The comet parameters (tail length , DNA% in tail ,tail moment) are then evaluated by a software .

In this study total of 100 individual cells were screened per subject (50 cells / each slide). Undamaged cells resemble an intact nucleus without a tail , while damaged cell has the appearance of a comet , it's parameter resemble estimation the degree of DNA damage .

Results and Discussion

It is known that sawdust to be a human carcinogen material with many health risks associated with exposure to them. It is a complex of physical, chemical and biological agents that make it difficult to detect specific allergy or irritation [23]. As the studies reporting DNA damage in carpenters are limited , in this study, the toxic effects on the DNA was evaluated in the carpenters who were exposed to wood dust that arise during the manufacture of wooden furniture . The investigation was conducted by implementation the comet assay. The level of DNA damage was determined as the percentage of cells with comets.

The results of comet assay of both groups were listed in table 1. Fifty exposed woodworkers and 50 control subjects were studied. In the present study, significant increase in comet parameters was observed in carpenters when compared to the controls using the comet assay. The strongest association of exposure to wood dust and development of DNA damage is observed in those craftsmen who were exposed to wood dust .

The exposed woodworkers, smokers or did not smoke cigarettes, have significant effect by comet test , showed statistically ($P < 0.05$) increase in DNA damage when compared with smokers and non-smokers of the control individuals. While, within the control group smokers showed significant($p < 0.05$) increase in comet parameters when compared with healthy non smokers , indicating DNA damage in the smokers

individuals meeting the results of other study correlate between smoking and DNA damage [1].

Table 1 : Alkaline Comet Assay Parameters for Wood Workers and Control Subjects.

Comet Parameters	Workers		Control Subjects	
	Smokers	Non- Smokers	Smokers	Non- Smokers
Tail length (px) (Mean ± SE)	40.15 ± 0.54 C	36.21 ± 0.43 C	12.81 ± 0.89 B	2.63 ± 0.33 A
Tail DNA % (Mean ± SE)	32.12 ± 0.87 C	29.56 ± 0.34 C	6.60 ± 0.06 B	2.29 ± 0.07 A
Tail moment (Mean ± SE)	12.90 ± 0.98 C	10.70 ± 0.10 C	0.846 ± 0.02 B	0.060 ± 0.03 A

•Similar letter in a column and row (for comparison between studies groups) mean there is no significant difference ($p < 0.05$), according to Duncan test.

The results of comet assay parameters revealed that there is a statistical significant increasing damage in the DNA of the exposed workers comparing with control group. ($P < 0.05$). Thus, levels of DNA damage in smoker wood's workers (tail length 40.15 ± 0.54 ; tail DNA % 32.12 ± 0.87 and tail moment 12.90 ± 0.98) and non-smokers (tail length 36.21 ± 0.43 ; tail DNA % 29.56 ± 0.34 and tail moment 10.70 ± 0.10) was significantly higher than in control group smokers (tail length 12.81 ± 0.89 ; tail DNA % 6.60 ± 0.06 and tail moment 0.846 ± 0.02) and non-smokers (tail length 2.63 ± 0.33 ; tail DNA % 2.29 ± 0.07 and tail moment 0.060 ± 0.03). Analysis of the data showed that the confounding factors (wood dust exposure) had a significant effect on DNA damage. Smoking did not have any significant effect on wood workers by comet test, despite smokers in exposed group showed higher comet parameters as seen in table 1. It is expected that the cause of this increase in the level of DNA damage between the timber factory workers is the constant exposure to wood dust during the furniture industry. These results showed sufficiently elevation of exposure to wood dust to cause such DNA damage and also the sensibility of the assay used. It appears that DNA damage was observed to be caused by the direct impact of toxic dust it selves. The finding of these results suggest that the wood workers exposed to an increase levels of genotoxicity, so they have an enhancement for cancer risk [13].

Overall, wood dust is a very common occupational exposure. All tested wood species so far causes inflammation, but varies in their potential. The present *in vitro* experiments suggests that wood dust possess a genotoxic potential independent of inflammation [17, 15]. Polish researchers suggested that workers who work in the factory of furniture had more DNA damage in lymphocytes rather than the control group [7]. The data supports an earlier study conducted by the authors which showed that there was DNA damage to the woodworkers is greater than controls [21].

The health troubles increased by the wood workers who don't wearing gloves or facemasks such as respiratory tract irritations, cough and asthma and infections of eye and skin . Low or lack of protective measures in terms of wood factories elucidate an increasing of genotoxicity in this study and illustrate an urgent need to educate workers about the occupational hazards that they are exposed to it through their work and the importance of wearing gloves and masks .Under the hazard communication standard of the Occupational Safety and Health Administration (OSHA), it is required to inform employees of the hazards of all hazardous chemicals and materials on the work-site. By familiarity the risk of a materials under use in the factory , employees are more able to keep themselves from this hazard and take preventative proceedings needful to work safely and safeguard their health . Even though, the control on dust resulted through the operations in the these factories is not assured, such operations have to supply with good local exhaust ventilation to reduce the genotoxicity of the wood dust [8].

As the important point in the role of carcinogen exposure for cancer risk is the DNA damage , this study suggest that comet assay is a good assessment way for the DNA damage caused by occupational exposure in the wood factories as a potential health hazards . The debatable results of different studies which are hard to be explained belong to the variation in study population in the culture , lifestyle, nutritional habit , sustenance and works in different regions under different climatic situations [19]. Extra inclusive planned studies that measure and followup the genotoxicity effects in wood factory are necessary to back these findings.

Conclusions

The deduce of this study suggest that the elevation in DNA damage appeared in the blood samples of wood furniture workers was due to wood dust exposure , and the usefulness of depending on the variations in the comet parameters as a marker for the DNA damage of workers engage in a wooden furniture factories .

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