




Comet assay as a sensitive technique in occupational health studies; A literature review

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Abstract

Background: Occupational health is a sensitive field in many countries where occupational exposure needs attention due to the lack of awareness. Several businesses, including leather, textile, iron, steel, and dyeing industries use a variety of mutagenic and genotoxic chemicals. Exposure to these chemicals may pose serious health risks, including genetic damage to workers. Comet assay is a technique that has been extensively used in assessing DNA damage in such workers.

Materials and methods: The literature search was done using PubMed and Google Scholar databases. Some studies were found reporting the use of comet assay as a sensitive and useful technique in assessing genetic damage in occupationally exposed workers. These studies have been presented in this paper to emphasize the importance and propriety of comet assay in assessing the genetic impact of hazardous exposures.

Result: Having gone through the available reports, the author of this paper found comet assay as a useful biotechnological technique in the field of occupational biomonitoring. This technique is a sensitive and cheap method for assessing genotoxicity.

Conclusion: Comet assay can be used as a reliable technique to quantify the impact of genotoxicants in the form of DNA damage at a single-cell level. This assay can be considered as a sensitive parameter whenever health assessment studies are to be designed.

Keywords: Comet Assay, DNA Damage, Occupational Health, Genotoxicity, Exposure, Health

Introduction

Occupational health is one of the priority areas in any country (1). Many countries spend considerable amounts of money and time to keep their workers safe. However, the field of occupational health is still ignored in many developing countries. In some parts of the world, workers are exposed to a number of hazardous chemicals, gases, and nascent metals due to the lack of protective equipment. The main reason behind the non-use of personal protection equipment (PPE) is the lack of awareness among workers and industrialists, which is crucial in safeguarding the health of workers (2). Countries caring for the health of their workers, assess their

health according to regular health and safety programs. Such programs are designed aimed at identifying various types of exposure threatening workers as well as their remedial measures (1).

Biotechnology is a field of science that deals with the scientific ways of identifying various chemicals that may have genotoxic effects on workers. Some methods have been developed for assessing or quantifying the effects of hazardous chemicals. Comet assay is one of the methods for assessing the genotoxic effects of such chemicals affecting the health of workers (3). Various businesses, including textile, dyeing, iron, steel, and leather industries use a variety of chemicals, including dyes and solvents,

which are genotoxic in nature (4–6). Any chemical that affects the integrity of deoxyribonucleic acid (DNA) may be considered genotoxic. Many dyes, such as azo dyes, have been considered genotoxic in nature (7). Such chemicals enter the cells and cause breaks in DNA, which may be single or double stranded (8). Comet assay can detect these breaks and quantify the amount of the broken DNA at the single-cell level. In the present paper, some attempts were made to review the use of comet assay in the field of occupational health. This review includes a number of studies, which have used comet assay in assessing DNA damage in the field of occupational health and safety.

Materials and Methods

A comprehensive electronic search was done to provide the present review. Relevant papers were

searched through PubMed and Google Scholar databases by different keywords, including 'comet assay' and 'DNA damage', and in various combinations with occupational exposure. All relevant freely available full-text studies and abstracts were included in this review. The retrieved studies employing comet assay in different industrial milieus (N=49) were originally published from 2000 to 2018. Besides, a total of 51 studies reporting comet assay in general (from 1988 to 2018) were retrieved. In total, 100 studies were collected for the review (Figure 1). The studies were then sorted based on their relevance to the present review. The exclusion criteria were applied at two levels of titles and abstract levels. After the careful meeting of both exclusion criteria, 31 most relevant studies were included in this review.

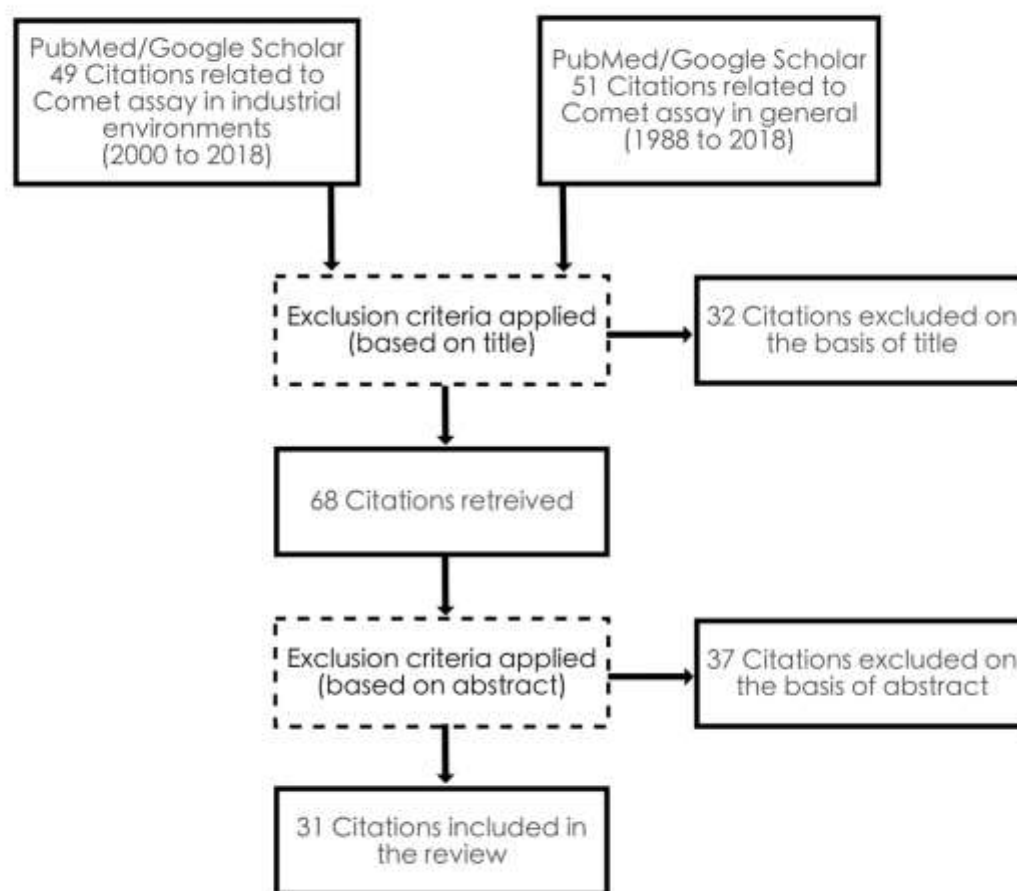


Figure 1: The flowchart of the methods used in retrieving the studies for the present review

Results

Occupational exposure to different chemicals, fumes, metal particulates, and gases may result in damage to DNA. Such effects are usually subclinical, unless cumulative changes lead to chronic health effects after long exposure. Metal exposure can induce different physiological

pathways in workers, thereby leading to a substantial disease pattern. For example, welding activities produce various gases, such as CO, CO₂, O₃, and fumes containing metals, including cadmium, chromium, and nickel, which are known mutagens and can lead to serious health hazards, such as cancer, after long-term exposure.

Various tools, including comet assay, the micronucleus test, sister chromatid exchanges, chromosomal aberrations, and the like have been used to assess DNA damage. Out of these tools, comet assay has emerged as a powerful biological tool, due to its applications in the fields of cancer research, chemical safety testing, as well as environmental and occupational studies. Comet assay, also known as single-cell gel electrophoresis (SCGE), is a method used for measuring DNA strand breaks in eukaryotic cells. It was introduced in 1984 by Ostling & Johansson (9) and later on modified by Singh *et al.* (10). Since that date, this technique has achieved popularity and has now been established as a standard technique for the evaluation of DNA damage, biomonitoring, DNA

repair, and genotoxicity testing. In this method, cells from subjects (for instance workers' lymphocytes) are embedded in agarose on a microscopic slide. These cells are lysed by applying a detergent and a high salt concentration to form nucleoids containing the supercoiled loops of DNA linked to the nuclear matrix. Electrophoresis is done at a high pH level. This results in the formation of structures on microscopic slides that resemble comets (Figure. 2). Comets are observed under a fluorescence microscope, using a fluorescent dye, such as DAPI or ethidium bromide. The DNA percentage in the tail region indicates the extent of the damage. Besides, the intensity of the comet tail relative to the head shows the quantity of DNA breaks (11).

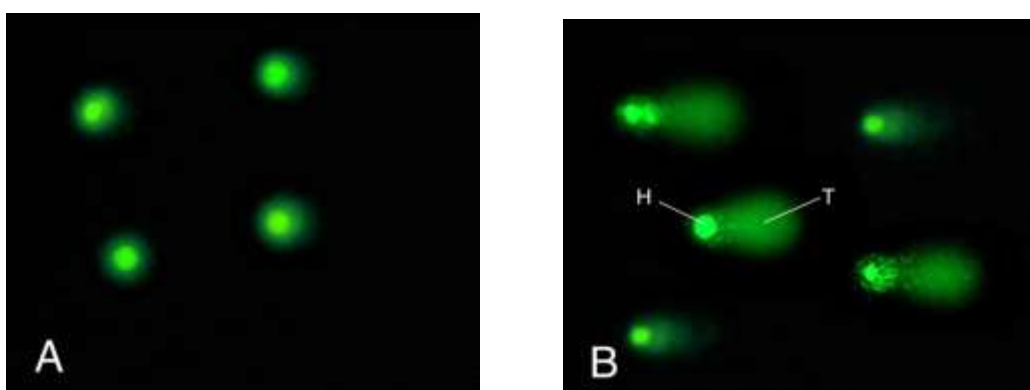


Figure 2: Comet assay slides showing the green coloured DNA of individual cells; (A) The comet assay slide of the undamaged DNA from four individual cells; round heads show the undamaged DNA. (B) The comet assay slide of the damaged DNA from five individual cells; a single comet is divided into a 'head' (H) and a 'tail' (T). The DNA present in the tail region of the comet indicates the extent of the damaged DNA (reprinted from www.cellbiolabs.com, with some modifications).

Due to its wide variety of applications, comet assay is utilized by many researchers to assess DNA damage in the workers of different industries. During the literature review, a number of health biomonitoring studies were found that used comet assay (12–16). Those studies were aimed at assessing the amount of genotoxicity caused by particular exposures in the workers of different industries, such as open-cast coal mining (17), the automotive industry (18), lead battery recycling plants (19), and the petrol industry (20). A study by Megyesi *et al.* (21) measured genotoxic DNA damage and assessed oxidative DNA damage caused by occupational exposure in groups exposed to benzene, polycyclic aromatic hydrocarbons, and styrene at the workplace so as to determine if comet assay could be used as an effective marker tool in genotoxicology (21). The study confirmed that occupational exposure could be identified using this method. Comet assay could be recognized as an excellent marker and a supplementary technique for monitoring the

presence or absence of genotoxic effects. Another study used comet assay to detect DNA damage in the blood lymphocytes of 30 workers exposed to asphalt fumes and 30 non-exposed controls (15). DNA damage was evaluated by the percentage of the DNA present in the comet tail (% tail DNA) in each cell. Results demonstrated that workers exposed to asphalt fumes suffered more DNA damage than the control group ($p < 0.01$). The present study showed that asphalt fumes caused a significant increase in the DNA damage, and that comet assay was a suitable method for determining the DNA damage in asphalt workers.

Another study evaluated the genotoxic potential of occupational exposure to perchloroethylene (PCE) in dry-cleaning workers. The study was carried out on 59 volunteers. The environmental monitoring of the exposure was carried out on personal breathing zone air samples collected during two consecutive working days by measuring the concentration of PCE air levels. The DNA damage detected by alkaline comet assay was significantly higher in dry-

cleaning workers than the controls. Comet assay was found to be a useful method for monitoring populations exposed to the low doses of PCE (22). Toluene is widely used in many industries. To estimate the genotoxic risk of toluene exposure, DNA damage was determined in the peripheral lymphocytes of 20 glue sniffers and 20 age-matched controls, using the alkaline comet assay (23). The increase in genetic damage in the sniffers was found to be statistically significant as against the control subjects ($P < 0.0001$).

Pesticides are known for their toxic effects. A study assessed DNA damage in the blood leukocytes of 29 Pakistani workers of a pesticide factory and 35 controls. The workers were exposed to various mixtures of organophosphates, carbamates, and pyrethroids. DNA damage was measured using the comet assay, by the mean comet tail length (μm) as the DNA damage index. The exposed workers were found to have had significantly longer comet tail lengths than the controls (24). Welding fumes have been classified as possibly carcinogenic to humans (Group 2B) by the International Agency for Research on Cancer (IARC). To assess the effects of welding fumes, DNA damage was examined in the lymphocytes of 30 welders and 22 controls, using comet assay. The results showed an increase in the level of DNA damage in the welders' lymphocytes compared to the controls, confirming the genotoxicity of welding fumes (25). Another study examined genotoxic effects in a population exposed to the coal residues of the open pit mine of "El Cerrejón". In the study, 100 exposed workers were divided into four groups according to different mining area activities, including the transport of extracted coal, equipment field maintenance, as well as coal stripping, and coal embarking. Blood samples were taken to investigate the biomarkers of genotoxicity, including the DNA damage index, the tail length, and the percentage of the tail DNA, using the alkaline version of comet assay in lymphocytes. All biomarkers showed statistically significant values higher in the exposed group than the non-exposed control group (17).

Discussion

After reviewing relevant studies, it was observed that comet assay had been used in assessing DNA damage in a number of occupation-centred studies. DNA damage was studied in peripheral lymphocytes in 60 workers occupationally exposed to trivalent chromium [Cr(III)] in a tannery, using comet assay (26). The subjects were divided into three groups of (i) exposure group I, including 30 tannery workers highly exposed to chromium working at the tanning department, (ii) exposure

group II, including 30 tannery workers with moderate chromium exposure working at the finishing department, and (iii) the control group, including 30 individuals with no exposure to physical or chemical genotoxic agents. The medians of the mean tail length (MTL) of the three groups were measured at 5.33 (2.90-8.50), 3.43 (2.31-8.29), and 2.04 (0.09-3.83) μm , respectively. The medians of the mean tail moment (MTM) were also measured at 6.28 (2.14-11.81), 3.41 (1.25-11.07), and 0.53 (0.13-3.29), respectively. The MTL and MTM of the two exposure groups were significantly higher than those of the control group ($P < 0.01$) (26). Using comet assay, Kianmehr *et al.* (27) carried out a study aimed at assessing the DNA damage level in peripheral blood lymphocytes (PBLs) in people working at bakeries fuelled by natural gas, kerosene, diesel, or firewood. In that study, 55 subjects were divided into four experimental groups and a control group. Each group was comprised of 11 members, based on the type of the fuel used. Using the Comet Score, i.e. the software for the DNA damage assessment, peripheral blood lymphocytes were examined. All bakers showed a significantly higher DNA damage level in peripheral blood lymphocytes than the individuals in the control group (27).

In the same vein, various animal models have been used to assess the effects of different genotoxicants. In this respect, some models, including rats, mice, and fish have been used to assess the effects of various hazardous chemicals, using comet assay. In a study by Dobrzyńska (28), DNA strand breaks in mouse somatic cells were examined. Male and female mice were repeatedly irradiated with X-rays and injected with nonylphenols for 2 weeks, 5 days a week. After the treatments, each animal's liver, spleen, femora, lungs, and kidneys were removed for comet assay. Nonylphenol induced DNA damage at different levels in different organs, having been varied by sex. In another study using mice as the model, comet assay was employed to detect genotoxicity caused by flumorph (29). The study examined the genotoxic effects of flumorph in the mice's organs, including the brain, liver, spleen, kidneys and sperms. Statistically significant increases were observed in comet assay in both dose-dependent and duration-dependent DNA damage levels in all organs assessed.

DNA damage is thought to be one of the mechanisms by which the cigarette smoke (CS) initiates a disease in the lungs. The potential of comet assay for measuring DNA damage in isolated rat lung alveolar type II epithelial cells (AEC II) was explored by Dalrymple *et al.* (30). Smoke-induced DNA damage can be quantified in isolated cells

following single or 5-day smoke exposure. This study also reported comet assay as a potential method for determining smoke or aerosol-induced DNA damage in AEC II isolated from rodents. Fish have been extensively used as models for comet assay in a number of studies. A recent study by Pandey *et al.* (31) examined the genotoxicity induced after in-vivo exposure to the freshwater fish, *Channa punctatus*, using comet assay and random amplified polymorphic DNA (RAPD). DNA damage was measured in erythrocytes in the form of the percentage of DNA damage in comet tails. Hence, one can conclude that comet assay is an important tool in DNA damage quantification in various experimental setups.

Conclusion

Single Cell Gel Electrophoresis, also known as comet assay, is used to detect DNA damage at the individual eukaryotic cell level. Comet assay has been widely used to measure the range of cellular responses to DNA damage. It has also numerous applications in genotoxicity studies, bio-monitoring, ecological testing, and human disease research. Comet assay is a simple, sensitive, and approved method for assessing genetic damage in various fields of research. In this review paper, different studies confirmed the efficacy of comet assay as a reliable method for determining DNA damage in different occupational health assessments. Comet assay assesses genetic instability in terms of the tail length, tail moment, percentage tail DNA, and intensity of tail DNA. Besides, this technique has been applied to different animal laboratories, including rats, mice, and fish to assess the impact of various hazardous chemicals. Many studies reviewed in this paper confirmed the aptitude and sensitivity of comet assay. In conclusion, occupational health studies can be designed so as to include comet assay as a sensitive and reliable method for measuring the DNA damage level to assess workers' "genetic health".

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Conflict of interest: None declared.

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