

Assessment of the deoxyribonucleic acid damage caused by occupational exposure to chemical compounds in Isfahan Polyacryl Company

Mahmoud Etebari, Abbas Jafarian-Dehkordi, Ahmad Kahookar, Shahla Moradi

Department of Pharmacology, Isfahan Pharmaceutical Sciences Research Center, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran

Background: Chemical pollutants found in industrial environments can cause chronic genotoxicity in vulnerable individuals during the long-term exposure. The primary purpose of the present study was to assess the deoxyribonucleic acid (DNA) damage caused by occupational exposure to industrial chemicals and secondary purpose is to investigate the effect of possible risk factors of genotoxicity.

Materials and Methods: The blood samples of the workers of Isfahan Polyacryl Company were evaluated in terms of genotoxicity using the comet assay method. The percentage of DNA in the tail and tail moment were measured and DNA damage was evaluated. Furthermore, the effect of age, smoking, duration of working in the company and working in two parts of the company on the degree of vulnerability to genotoxicity was assessed. **Results:** The amount of DNA damage in the target group (the production line workers) was significantly higher than the control group (the staffs), 3.87 versus 1.52 as tail moment, ($P < 0.0001$). DNA damage was significantly higher in smoker groups compared with non-smoker target group and control group, 4.18 versus 3.07 and 1.52 respectively as tail moment, ($P < 0.0001$). Furthermore, it was higher in person working in two different parts of the company compared to those work in one part and control group, 4.63 versus 3.74 and 1.52 respectively as tail moment, ($P < 0.0001$). **Conclusion:** Occupational exposure to Polyacryl caused DNA damage. Smoking and working in two parts of the company may have a significant role in DNA damage.

Key words: Comet assay, deoxyribonucleic acid damage, genotoxicity, Isfahan Polyacryl Company, occupational exposure

How to cite this article: Etebari M, Jafarian Dehkordi A, Kahookar A, Moradi S. Assessment of the deoxyribonucleic acid damage caused by occupational exposure to chemical compounds in Isfahan Polyacryl Company. J Res Med Sci 2014;19:542-8.

INTRODUCTION

The workers of different industries are exposed to various chemicals with potential peril of deoxyribonucleic acid (DNA) damage. Some of these agents may even increase the risk of different cancers, both in the workers and in those who are passively exposed to them due to environmental pollution.^[1] The workers with chronic contact with toxic compounds and other chemicals must be controlled periodically in terms of DNA damage and genotoxicity. Although the concentration of the perilous chemicals and other toxic agents must always be strictly kept below the threshold limit values, chronic toxicity may occur in workers with a higher degree of vulnerability.^[2] It is, therefore, essential to monitor the workplace conditions as well as the workers health status. Environmental monitoring has long been used to control the working conditions concerning chemicals and toxic agents. This method is rather cheap and non-invasive, but not sufficient to assess the possible dangers of chronic exposure to legally acceptable levels of the chemicals.^[2] DNA damage may occur by physical

or chemical agents can lead to mutations as well as increasing the risk of new genetic disorders. Hence, evaluation of the possible genotoxicity caused by these agents is most important.^[2] Different methods have been developed so far to fulfill this goal. The cytogenic and immunologic methods have been widely studied. Being costly and time consuming, these methods seem less practical today and are giving way to other simpler and more economical techniques. Besides, the mentioned methods are only applicable on cells within the reproduction and multiplication phase, which imposes another limitation on the control process. Comet assay is the alternative method for DNA damage monitoring, which is simple, sensitive and economical enough to be used as a routine genotoxicity test. All the mentioned advantages have highlighted this technique as a standard method for DNA damage assessment in the past decade.^[3-7]

Comet assay, also known as a single cell gel method and micro gel electrophoresis, was first introduced as a micro electrophoresis method for direct observation of DNA damage by Johanson and Ostling in the 1984.^[1,8,9]

Address for correspondence: Prof. Mahmoud Etebari, Department of Pharmacology, Isfahan Pharmaceutical Sciences Research Center, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran. E-mail: etebari@pharm.mui.ac.ir

Received: 03-03-2013; **Revised:** 13-10-2013; **Accepted:** 21-01-2014

This method, however, underwent several corrections and alterations, as those made by Faust *et al.*, to turn into one of the most convenient and sensitive techniques for DNA damage assessment.^[10] The mechanism by which comet assay detects DNA damage was first explained by Reydberg and Johanson. Based on their explanation, the cells trapped in the agarose gel and lysed under the alkaline pH, find the possibility of presenting a little opening in the DNA. Under the effect of the electrical flow, the DNA molecules then move toward anode, to form the comets. The comet formation pattern is determined by two important factors; the size of the DNA fragments and the number of broken ends.^[11] As the percent of damage increases, the free DNA fragments move in the tail and in more severe cases, the head and the tail separate completely. Comet assay can be used to differentiate the single and double strand breakages. This is fulfilled by applying neutral or alkaline lysis. Since this method is for DNA damage assessment in single cells, it is essential to conduct the test in a way that the separation of the cells would be possible.^[12] To perform the test, suspension of the separated cells should be prepared. DNA damage should be assessed in the cells without giving them the opportunity of being exposed to new genotoxic compounds or of repairing the previously made damages.^[12] Human lymphocytes are the cells commonly used for the assay.^[12] The cells are usually suspended in agarose to form a final concentration of 0.5-1% in 35-45°C and then transferred on normal or serrate slides. It is important that the gel be totally solidified before continuing the rest of the assay. The slides are then placed within the lysing buffer to lyse the available cells.^[13] Should a neutral lysing buffer be used, double strand damage detection will be possible. In case that single strand damage assessment is required, an alkaline lysing buffer is needed.

The time and voltage necessary for the electrophoresis depends obviously on the level of the DNA damage and the salt concentration within the electrophoresis buffer. Since microscopic observation of DNA migration is possible with even a portion of a millimeter of DNA movement, the essential DNA migration which leads to comet formation requires a short electrophoresis time (5-30 min) and a low voltage (0.5-5 v/cm). Following the electrophoresis, the slides are washed and dyed with a DNA binding fluorescent dye.^[13] The comets can then be viewed using a fluorescent microscope.

The resulted comets can be assessed in different ways. One of the simplest techniques used for this purpose is to rank the resulted comets visually.^[14] However this is not a very reliable and sensitive method to be used regularly. Another method frequently used to assess the amount of damage is measuring the distance between the DNA and the nucleus. Computer-based analysis of the resulted comets using available software heightens strongly the sensitivity of the

assay.^[10,14-16] The final conclusions are made based upon two easily calculated factors; tail moment which is a combination of the tail length and the total available DNA in it and % DNA in the tail which determines the ratio of the normal fragments over the broken ones.^[17]

The purpose of the present study was to assess the DNA damage caused by occupational exposure to industrial chemicals in Isfahan Polyacryl Company workers and to investigate the effect of possible risk factors believed to predispose them to genotoxicity on the amount of DNA damage, using comet assay.

MATERIALS AND METHODS

Study groups

The target group

Members were selected among the workers of the production line in Isfahan Polyacryl Company, on the basis of filled questionnaires including personal and occupational information, record of service, working section or department and health and medication record.

The negative control group

Members were selected among the non-smoker office staffs of the same company with no record of exposure to chemicals of the production line, regardless of their age and based upon the same procedure used for the target group selection.

Sample preparation

Lymphocytes were isolated from whole blood samples that has been diluted and treated with an anti-coagulant agent.

Alkaline comet assay

Alkaline comet assay procedure has been described in our previous studies.^[18-21] Briefly, cell suspensions (1×10^6 cells/ml) were mixed with of 1% low melting point agarose (37°C) and were placed on the precoated slides (1% normal melting point agarose). Slides were covered with cover glasses for 5 min. H_2O_2 (200 μ M) were added to the positive control slides for 20 min. The slides were incubated with lysis solution (pH = 10.0) for 40 min. Afterward, the slides were rinsed with distilled water to remove excess lysis solution. In the next step, the slides were incubated with electrophoresis buffer (pH > 13.0) for 40 min. Samples were electrophoresed for 40 min at 25 V with an electricity current adjusted to 300 mA.^[16,17] After electrophoresis, the slides were rinsed with distilled water to remove excess alkaline buffer and were placed in neutralization solution (pH = 7.5) for 10 min. Each slide was subsequently covered with dye solution (20 μ g/ml ethidium bromide) for 5 min and washed with distilled water. The comets were visualized

under $\times 400$ magnification using fluorescence microscopy with an excitation filter of 510-560 nm and barrier filter of 590 nm.^[16,17] All stages in comet assay were performed at 4°C in dark conditions and all solutions were cool.

Statistical analysis

One-way analysis of variance (ANOVA) was used to compare the results of comet assay, followed by Tukey's multiple comparison *post hoc* tests, using Graphpad Prism V 5.0 (Graphpad Software, San Diego, CA) software. $P \leq 0.05$ were considered as statistically significant.

RESULTS

Comet assay results of workers in different parts of the company

To assess the genetic toxicity caused by occupational exposure to the chemicals and other toxic agents in Isfahan Polyacryl Company, the amount of DNA damage in the workers' lymphocytes both in the production line (target group) and the staffs (control group) was determined and compared using the comet assay. A total of 120 workers were interred in this study, 60 of them as a control group and others as experimental groups. All workers were men, the average of their age was 35.85 ± 4.4 years and the average of exposure duration was $13. \pm 1.2$ years. Two critical factors including the percentage of DNA in the tail and tail moment ($\% \text{ DNA in tail} \times \text{tail length}$) were calculated. Based on these criteria, the comparison made between the workers of different sections along with staffs showed a significant difference which means that the people working in the production line suffered from greater DNA damage than the office workers [Figure 1]. The result of the one-way analysis (ANOVA) for the percentage of DNA in the tail of studding groups was significant ($P < 0.0001$). According to the results of the Tukey's multiple comparison *post hoc* test, working in all studding parts of the company increased the percentage of DNA in tail significantly ($P < 0.001$) compared with the control group [Figure 1a]. One-way analysis result of the tail moment for all groups was significant ($P < 0.0001$), more over the results of Tukey's multiple comparison *post hoc* test for all groups showed a significant increase ($P < 0.001$) in this parameter compared with the control group [Figure 1b].

Comet assay results of workers with one and two jobs in the company

Working in two different parts of the company is one of the other predisposing factors for some cases. According to the extracted information, about 7% of the production line workers were working in two parts. The result of the one-way analysis (ANOVA) for the percentage of DNA in the tail was significant ($P < 0.0001$). According to the results of Tukey's multiple comparison *post hoc* test, working in one and two parts of the company increased the percentage

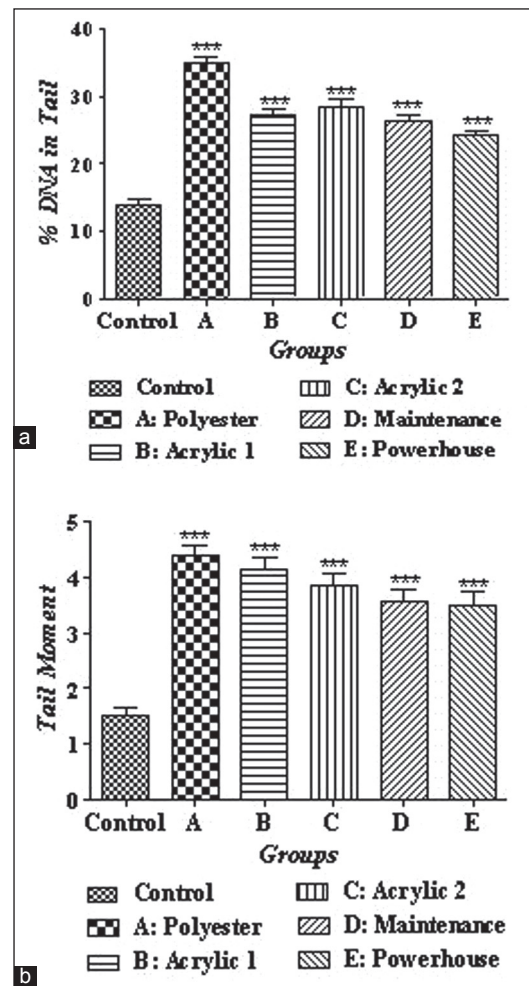


Figure 1: (a) Comparison of % deoxyribonucleic acid in tail and (b) tail moment of the workers in different parts of the company. Each graph has been represented as mean \pm standard error of mean for 50 workers

of DNA in tail significantly (both $P < 0.001$) compared with the control group, more over a significant difference ($P < 0.05$) was seen between one and two-job worker groups [Figure 2a]. One-way analysis result of the tail moment was significant ($P < 0.0001$). The results of Tukey's multiple comparison *post hoc* tests for this parameter were similar with the result of the percentage of DNA in tail [Figure 2b].

Comet assay results of the smoker and non-smoker workers

Smoking is one of the personal risk factors may predispose the workers to possible genotoxicity. Based on the pre-filled questionnaires, 11.6% of the total studied population used to smoke cigarettes. The percentage of DNA in the tail and tail moment ($\% \text{ DNA in tail} \times \text{tail length}$), were calculated for these workers and showed a significant difference in the one-way analysis (ANOVA) results ($P < 0.0001$). Moreover, the Tukey's multiple comparison *post hoc* test results showed significant ($P < 0.001$) increase in the percentage of DNA in the tail for both smoker and non-smoker groups in comparison with the control group while comparison of

this parameter in smokers with non-smoker was significant ($P < 0.05$) [Figure 3a]. The One-way analysis of the tail moment data showed significant ($P < 0.0001$). Tukey's multiple comparison *post hoc* tests showed both groups had significant difference with the control group and with each other (respectively $P < 0.001$ and $P < 0.01$) [Figure 3b].

DISCUSSION

A great number of workers are exposed to different toxic agents with potential genotoxic effects.^[1] Since these workers have chronic contact with these compounds and are thus exposed to potential DNA damage, it is essential that they be periodically assessed using reliable biomarkers such as comet assay, sister chromatic exchange, micronucleus. Many of these tests, however, are relatively expensive and complicated due to the need for cell culture and therefore, are not convenient for monitoring of a big working population.^[22] Nevertheless, being rather simple, reliable and relatively less costly, comet assay is one of the most convenient methods to be used for DNA damage assessment in different

industrial workplaces.^[17] This method also enables us to detect different types of DNA damage and to observe these damages directly in each and every of the damaged cells.^[23-25] Besides, the possibility of data collecting from the cell surface which provides us with strong statistical analysis, the need for the limited number of sample cells for the analysis (<10000) and the possibility of using all kinds of cells with nucleus both *in vitro* and *in vivo* mark it as a very convenient method for this purpose.^[22,26,27] Comet assay can also enable us to clinically control the DNA-damage-inducing disorders or treatments in a short span of time.^[10,22] One of the other advantages offered by comet assay is its high differentiating capacity which minimizes the probability of attaining unreal positive results.^[17] It has been shown that results obtained from comet assay are in most of the cases compatible with the results acquired from cytogenetic tests,^[28,29] which proves that not only does the method possess enough sensitivity to detect even the smallest damage in DNA, but also shows negative results whenever lack of damage cannot be detected by other tests. That's why Faust *et al.* believe that the results attained from comet assay are hopeful bioassay indexes to be used for controlling the workers exposed to industrial and non-industrial genotoxic agents.^[10]

Different factors can impact a worker's vulnerability to DNA damage. These factors include workplace, work time, record

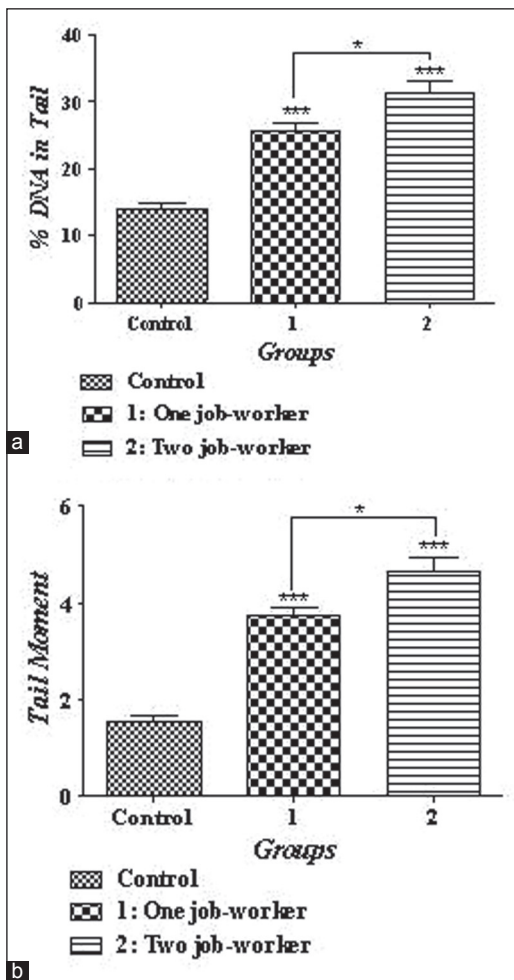


Figure 2: (a) Comparison of % deoxyribonucleic acid in tail and (b) tail moment of workers with one and two works in the company. Each graph has been represented as mean \pm standard error of mean for 50 workers

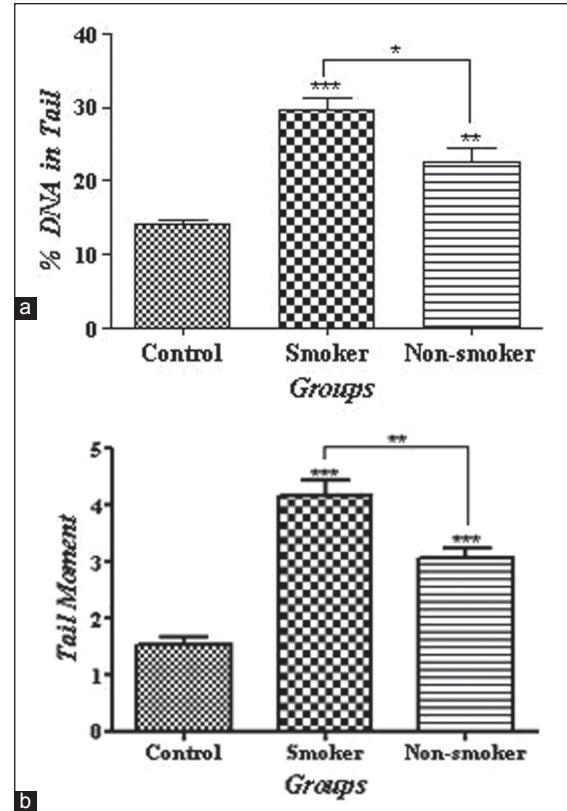


Figure 3: (a) Comparison of % deoxyribonucleic acid in tail and (b) tail moment of the smoker and non-smoker workers in all parts of the company. Each graph has been represented as mean \pm standard error of mean for 50 workers

of service, occupational stress, vocational pressure, type of chemicals available in the workplace, life style, diet, physical activity, smoking, personal health and even age and sex.^[10]

In this research, the office workers of Isfahan Polyacryl Company were considered as the control group, whereas the production line workers served as the target group. The data from the target group were collected from five different company workshops, including polyester, acrylic 1 and acrylic 2 production lines, maintenance department and the Power house. As shown in Figure 1, the highest amount of DNA damage belongs to the workers of polyester workshop and the lowest belongs to powerhouse workers for whom the two calculated parameters (% DNA in the tail and tail Moment) offer significant differences for all groups compared with the control group. This significant DNA damage observed in the production line workers can be caused by chemicals such as organic solvents, toluene, chloroform, ethanol, arsenic, chrome, acrylonitril, benzene and so on, whose genotoxic effects have long been established in numerous studies.^[30-38]

It has also been observed that people working in two different workshops demonstrate a higher amount of DNA damage, compared with those working only in one single section [Figure 2]. Based on departmental analysis, 2.9% of the workers in the polyester production line, with as much as 31.63% of DNA damage, work in two different company sections. This figure increases to 25% in the acrylic 2 section, by 28.62% of DNA damage came in the second rank after polyester workshop. For Acrylic 1 by 28.43% of DNA damage, the figure equals 13.6%. The analysis shows that the amount of DNA damage in the Maintenance department workers is as much as 27.77% which is probably so high since a lot of people working there work in other sections as well. The fact that 44.5% of the workers of maintenance section work in one of the other workshops signifies that working simultaneously in two different sections can be considered as a predisposing risk factor to DNA damage. In this case, for instance, if the workers with two jobs are not considered, the percent of DNA damage for the maintenance department will decline to 24.6%.

Another risk factor evaluated in this study was smoking, which has also been taken in to consideration in some of the previous bioassay studies.^[17] Cigarette and its derivatives are complex mixtures of substances with potential for genotoxicity.^[10] In this research, factors such as smoking and its record were included. According to an investigation made in Italy, smoking can cause a 10% increase of % DNA in the tail in smokers in comparison with a non-smoker population. Nevertheless, no significant relationship was found between DNA damage percent and the number of cigarettes smoked per day.^[39,40] Another study

conducted among people with two different age-range averages^[23,24,41-47] revealed that smoking caused up to 40% increase in DNA damage percentage both in the youngsters and in the elderly.^[15] On the contrary, some investigations, mostly made in working environments, suggested that smoking may have little effect on vulnerability to DNA damage.^[48,49] The present study, however, confirms that the percentage of DNA damage in smoking workers is considerably higher than that of the non-smoking working population [Figure 3]. The latter part was confirmed by the results attained from other studies.^[39,40,50] This can, of course, occur since due to the rapid intra-cell DNA repair, comet assay can just detect the amount of damage recently done to DNA.^[50,51] In a study by Howard *et al.* revealed that no significant difference in DNA damage exists between those who quit smoking more than a year before (ex-smokers) and those who never smoked.^[40,52]

CONCLUSION

Although most of the cases of DNA damage are repaired, some of them may persist over time hence that the negative outcomes appear much later and even after the end of the workers' contact with hazardous chemicals. This study showed that working in two different parts of the company and smoking are two factors of increasing DNA damage of workers. It is, therefore, necessary to control the workers, every once in a while, in terms of DNA damage and genotoxicity using a convenient bioassay method such as comet assay. Periodic review of the workers' health and amount of the chemical compounds in industrial environments is necessary.

ACKNOWLEDGMENTS

This paper was derived from a pharmacy doctorate thesis (no. 385302) in Isfahan University of Medical Sciences, Isfahan, Iran. We would like to acknowledge the research Department of Isfahan University of Medical Sciences, Isfahan, I. R. Iran, for their co-operation and financial supports.

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Source of Support: We would like to acknowledge the Research Department of Isfahan University of Medical Sciences, Isfahan, I. R. Iran, for their Co-operation and Financial Supports, **Conflict of Interest:** None declared.