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genotoxicity produced in the epithelial cells of the oral cavity and Comet assay in urinary tract of workers in aluminum factories

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ABSTRACT

Whilst being environmentally abundant, aluminum is not essential for life. On the contrary, aluminum is a widely recognized neurotoxin that inhibits more than 200 biologically important functions and causes various adverse effects in plants, animals, and humans. The acute toxicity of aluminum is low. No acute effects due to dietary exposure to aluminum have been observed in the general population. A compendium is provided of aluminum compounds used in industrial settings, and as pharmaceuticals, food additives, cosmetics and as other household products. In our current study, the genotoxicity of aluminum was evaluated in epithelial cells by Micronucleus assay in buccal cells and Comet assay in urinary tract cells, 50 samples were taken from the workers in the aluminum factory and 50 samples as control. The results showed significant increased in the average of the affected cells (8.86 ± 1.36) and the average total cell total (51.66 ± 18.19) in urinary tract while the average of damage cells (72.02 ± 8.99) and It is noted that the mean micronuclei (1.61±29.80) and the average abnormality in all cells (3.06 ±92.54) while the Mni(51.28 ±10.61). These results suggest that the estimation of cellular genetic damage in epithelial cells by Micronucleus assay and Comet assay estimation is important to know the toxic effects of aluminum.

Introduction

Aluminum (Al) is abundantly distributed in our environment, and compounds containing Al have been used in manufacturing (e.g., clays, glasses, and alum) for centuries. Despite its abundance, Al was first isolated as an element in 1827, and its use as being a silvery metal began only after 1886. Al is a new metal in this context. Because of its beneficial characteristics such as a lightweight, nonmagnetic, malleable, and ductile element, Al has a widespread and important use in industrial applications and consumer products. Al is also used in cooking utensils and in pharmacological agents including antacids and antiperspirants from which the element enters the human body.[1]. Occupational exposure to aluminum occurs during the refining of the primary metal and in secondary industries that use aluminum products. Several studies have reported adverse respiratory tract effects in aluminum industry employees. Asthma-like symptoms, known as potroom asthma, have been the most intensely investigated respiratory effect.[2]. aluminum salts as well, which they believe was induced by diphtheria toxoid, TT and pertussis (DTP) vaccination. Similar cases of aluminum contact sensitivity demonstrated by positive reactions to aluminum chambers and aluminum salts were reported in children and adults by Akyol et al.,2004 [3]. The adsorption of antigens on poorly soluble aluminum hydroxide augments the immunological effect [4].The primary organ for aluminum elimination is the kidney, which is believed to eliminate > 95% of excreted aluminum. Dietary intakes of 3.5 to 11.5 mg Al/day result in a daily excretion of 4 to 12 µg [5]. Aluminum also binds to the phosphate groups of nucleoside di- and triphosphates, such as ATP and can thus influence energy metabolism. Furthermore, Al inhibits the functions of various protein kinases and phosphatases [6]. About 92% of human cancers are derived from the external and internal epithelial cells, that is, the skin, epithelial cells, and epithelial cells of the gut lining[7]. Epithelial cells of the lining of the mouth represent a preferred target site for early genotoxic

events due to carcinogens and hereditary toxic agents entering the body through inhalation and ingestion [8]. The lining of the mouth continuously maintains itself by rejuvenating the cells as it produces new cells in the basal layer by mitotic division[9].

Materials and methods

Genotoxic effects of aluminum factory , As it was taken 50 samples from the workers in the aluminum factory and 50samples as control, using Micronucleus assay in buccal cells and Comet assay in urinary tract cells.

Single-cell gel electrophoresis (comet assay):

We adopted a standard protocol for the comet assay preparation and analysis. We performed the comet assay under alkaline conditions (pH 12.6), which detect double- and single-strand breaks and alkali-labile sites[10].We prepared the slides by mixing 5 µL of homogenate cells prepared (homogenized in 20 vol [wt/vol] of STM buffer [sucrose 250 mmol/L, Tris-HCl 50 mmol/L, pH 7.4, MgSO4 5 mmol/L, phenylmethylsulfonyl fluoride 0.5 mmol/L]), with 95 µL of low melting point agarose (0.75%) for blood samples or 80 µL for hippocampus samples. We added the mixture (cells/agarose) to a fully frosted microscope slide coated with a layer of 500 µL of normal melting agarose (1%). After solidification, we removed the cover slip and placed the slides in a lyses solution (2.5 M NaCl, 100 mM EDTA and 10 mM Tris, pH 10.0–10.5, with freshly added 1% Triton X-100 and 10% DMSO) for 1 day.

Subsequently, we incubated the slides in freshly made alkaline buffer (300 mM NaOH and 1 mM EDTA, pH12.6) for 10minutes. The DNA was electrophoresed for 20 minutes at 25 V (0.90 V/cm) and 300 mA under alkaline conditions (pH 12.6). After that, we neutralized the slides with 0.4 M Tris (pH 7.5). Finally, we stained the DNA with Cybergreen stain. We used negative and positive controls for each electrophoresis assay to ensure the reliability of the procedure[11].

Micronucleus assay in exfoliated buccal cells:

The samples were collected from individuals working in aluminum factory in Baghdad. The test was performed according to the method described by Gopal and Padma, 2018 [12] as follows: People were asked to wash their mouth with water and using a sterile dry polypropylene cotton swab to scrape cells from the lining of each cheeks and pressed on a clean microscope slide. It was dried with air and fixed with methyl alcohol. It was stained by May-Grunwald and then dyed with giemsa. The frequency of micronuclei was recorded as 2000 cells per capita were recorded in each case to determine the percentage of MN.

Results and discussion

Determination of DNA damage in the epithelial cells of the urinary tract in individuals working in aluminum factories with comet assay technology:

Table 1 and figure 1 show Evaluate the mean differences of the affected DNA cells . significant differences $p \leq 0.05$ were noticed in control and workers of aluminum factory.

Table 1: Percentage values of cells by DNA damage of aluminum workers

treatment $Mg.kg^{-1}.b.wt$	Anomalies SD ± M	Average of damage anomalies SE ± MD	Average of damage cells SD ± M	Average of damage total cells SE ± MD
Control	57.33 ±7.05	-	123.68 ± 128.34	-
Workers	48.48±6.53	*8.86± 1.36	72.02 ± 8.99	* 51.66 ± 18.19

* Significant at the P< 0. 05 (Independent Samples Test), M Average test, S.D standard deviation, MD average differences, S.E standard error

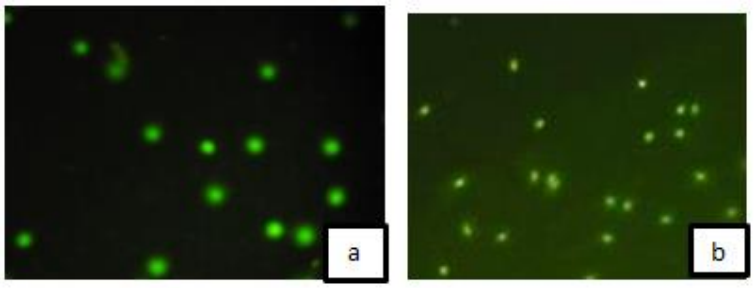

Figure 1: Different levels of DNA damage in epithelial cells Lining of the urinary system for aluminum workers a: Cells with DNA not affected by the negative control group,b: Cells with DNA damaged by the working group 100 X Cybergreen stain

This test is used to assess the risk of factors or substances that have the potential for genetic toxicity or mutations. They help detect DNA damage and detect a wide range of primary DNA lesions that cannot be identified by any other tests. This test applies to a wide range of tissues or any cell types being sensitive to a low level of DNA damage and requires only a small amount of cells per sample and can be completed in a short period of time [13]. In addition, there is a list of many environmental and industrial pollutants found in low doses can cause kidney damage if used in high doses; some toxic agents that are poorly found in any dose and are harmful are able to cause renal injury, and the kidney disease problem increases. Due to the incorrect use of some treatments [14].

This test is a valuable experimental tool designed to map DNA damage to human cells in vivo for environmental and professional monitoring, as well as for therapeutic purposes, such as pre-transplant storage, during tissue engineering, and in previous in vivo experimental assays. Moreover, because of their great diversity, the comet test allows exploring the use of alternative cell types to assess DNA damage, such as epithelial cells because epithelial cells form many organs and have the potential to serve as biomaterials that can be used to assess genetic toxicity and can also be biological markers for early impact [15]. 80% of cancers are of epithelial origin, suggesting the importance of studying DNA damage in these tissues. In fact, studies including comet testing in epithelial cells have clear clinical applications and help in examining genetic toxicity within the body and in laboratory studies [16]. Pazy-Mino et al.[17] showed that indicating of DNA damage caused by aluminum ions While, Toimela et al.[18] did not found cytotoxic effects of aluminum in epithelial and neuronal cell line ,respectively. There are several causes have been proposed to explain cyto- toxicity production by aluminum

Determination of Micronuclei in Oral Epithelial Cells in Individuals Working in Aluminum Factories:

The results showed an increase in the frequency of Micronuclei in the epithelial cells of the group of workers when compared to the basic level represented in the repeat of the Micronuclei of the control group as shown in the table 2 and figure 2 show Cellular genetic damage in the exfoliated buccal cells of epithelial cells were evaluated for the factories of Aluminum workers.

Table 2: The mean differences of the frequency of the micronuclei in the in exfoliated buccal cells of epithelial cells which evaluated for the factory of Aluminum Workers

Treatment Mg.kg^{-1}.b.wt	MNi MD ±SE	**Anomalies** MD ±SE	MNi M ±SD	**Anomalies** M ±SD
Control	–	–	81.38 ± 3.50	100.32 ± 7.05
Workers	*29.80 ± 1.61	*92.53 ± 3.06	51.28 ± 10.61	20.39 ± 192.96

* Significant at the P< 0. 05 (Independent Samples Test), M Average test, S.D standard deviation, MD average differences, S.E standard error

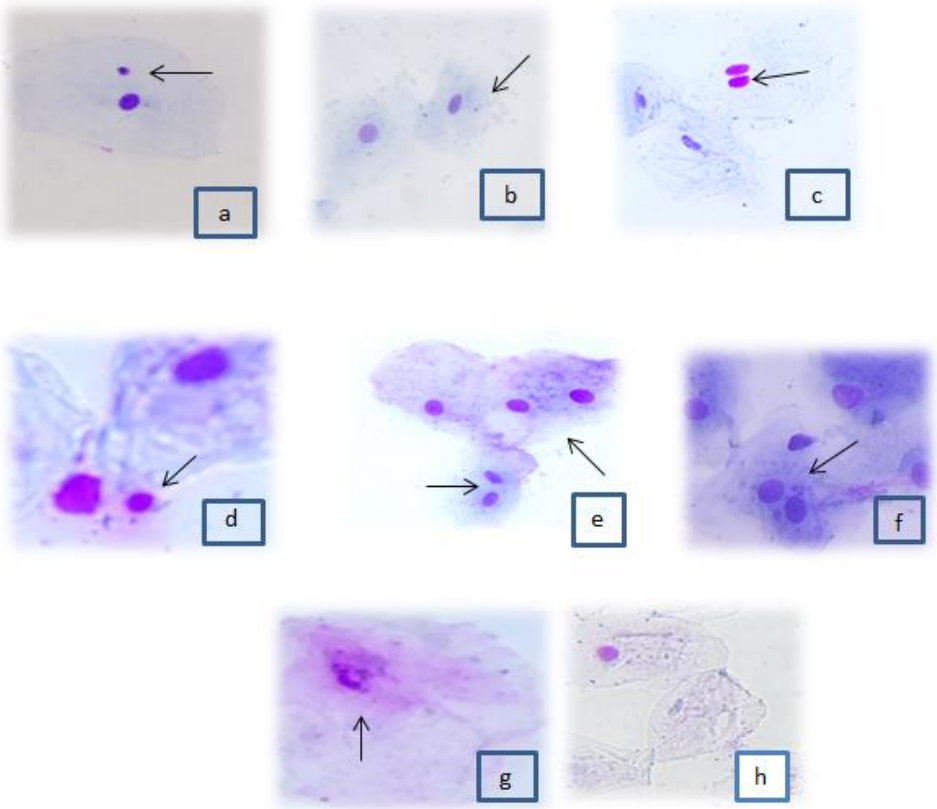

Figure 2: Cellular genetic damage in the exfoliated buccal cells of epithelial cells were evaluated for the factories of Aluminum workers A: micronucleus b: cell with condenced chromatin Binucleated cell d: Broken egg c,e:
h:Karyolysis cell g: karyorrhetic cell f : Binucleated cell with Micronucleus
Maygrünwald + Gemsa stain 40X

The examination of micronuclei performed on the epithelial cells of Oral is to detect biomarkers of early biological effects[19]. Test of the micronuclei of epithelial cells is a cellular technique for measuring DNA damage and signs of cell death in the oral epithelium [20]. Epithelial cells of the endothelium form the first barrier to inhalation or ingestion and are able to metabolize carcinogens near reactive products [21]. The possibility of decomposition or degeneration in a cell in the form of condensed chromatin, pyknotic, loss of nuclear materials karyolitic, which is in the form of the so-called ghost[20].In rare cases, some cells can also appear with two nuclei in the same cytoplasm stage, or form a nuclear bud or broken egg or form small nuclei (MN) near nuclei in the same cytoplasm. These biomarkers for DNA damage (eg, MN, nuclear buds) and cell death (such as apoptosis, karyolysis) can be observed in epithelial cells, and through it can assess the genetic toxicity and effects of cell inhibition[8].

The percentages of cellular genetic damage detected are as follows as shown in table 3:

Micronucleu:

The percentage of micronuclei in buccal cells of individuals working in aluminum factories (25%). Micronucleus are mainly produced by undeveloped chromosome fragments or whole chromosomes during mitosis because they fail to adhere properly to the spindle during chromosomal isolation [21].

Binucleation: Percentage of Binucleation cells in buccal cells of the aluminum factories was 20.8% compared to the control group.

Condensed chromatin cells: Percentage in buccal cells of individuals working in aluminum factories (4.6%).

pyknotic cells: Percentage in buccal cells of individuals working in aluminum factories (4.9%).

karyolysis cells: Percentage in buccal cells of individuals working in aluminum factories (27.3%).

Karyohexis cells: Percentage in buccal cells of individuals working in aluminum factories (8%).

Broken egg: Percentage of buccal cells in Individuals Working in Aluminum Factories (9.6%).

Table 3: Percentages of micronuclei of the exfoliated buccal cells of epithelial cells were evaluated for the factories of Aluminum workers

Anomalies	Control	Workers
Micronucleus%	0.21	25
Binucleation%	0.16	20. 8
Condensed chromatin%	0.08	4. 6
Pyknosis%	0.09	4.9
Broken egg%	0.09	9.6
Karyohexis%	0.08	8
Karyolysis%	0.24	27.3

Oral cells are in contact with environmental conditions, because oral epithelial cells are an important target for inhalation poisoning, and there are sufficient reasons to expect that they show evidence of genetic toxicity.

We conclude from this study that estimating cellular genetic damage in epithelial cells of oral cavity and urinary tract by estimating micronucleus assay and comet assay is important to know the toxic effects of treatments on humans**.**

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الكشف عن السمية الوراثية الناتجة في الخلايا الطلائية لتجويف الفم وبطانة المثانة للعاملين في مصانع الالمنيوم

سراب دلف خلف ، وجدي صبيح صادق

قسم علوم الحياة ، كلية العلوم ، جامعة تكريت ، تكريت ، العراق

الملخص

إن الألومنيوم ليس ضروريًا للحياة على الرغم من وفرته في البيئة وله سمية عصبية على نطاق واسع ويمنع أكثر من 200 وظيفة مهمة بيولوجيا ويسبب العديد من الآثار الضارة في النباتات والحيوانات والبشر, وتكون سميته الحادة منخفضة اذ لم يلاحظ أي آثار حادة عند التعرض للألمنيوم, يستخدم الألومنيوم في البيئات الصناعية، وفي صناعة الأدوية ومضافات غذائية ومستحضرات تجميل وغيرها من المنتجات المنزلية. وفي دراستنا الحالية، تم تقييم السمية الوراثية للألمنيوم في الخلايا الظهارية لتجويف الفم بواسطة اختبار تقدير النوى الصغرى وتم تقدير تلف الدنا DNA بواسطة تقنية تقدير الهالة لبطانة المثانة، وتم أخذ 50 عينة من العاملين في مصانع الألمنيوم و 50 عينة للسيطرة, أظهرت نتائج هذه الدراسة أن متوسط الخلايا المصابة (8.86 ± 1.36) ومتوسط إجمالي الخلايا الكلية (51.66 ± 18.19) في المثانة, بينما بلغت معدل الخلايا المتضررة (72.02 ± 8.99). ويلاحظ أن متوسط النوى الصغرى (1.61 ± 29.80) ومتوسط الشذوذ في جميع الخلايا (3.06 ± 92.54), وبلغت النوى الصغرى(51.28 ± 10.61) . تشير هذه النتائج إلى أن تقدير الأضرار الوراثية الخلوية في الخلايا الظهارية بواسطة اختبار النوى الصغرى وتقدير الهالة مهم لمعرفة الآثار السامة للألمنيوم.