OXIDATIVE STRESS AND GENOTOXICITY USING ALKALINE COMET ASSAY AMONG ANESTHIOLOGISTS

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ABSTRACT

Introduction: Long-term inhalation anesthetics exposure may provide health risks that cannot be completely ruled out**. Aim of the study:** The target of this research was to see how waste anesthetic gases influenced DNA damage and oxidative damage. **Subjects and Methods:** Two groups of healthcare workers from the department of anesthesiology joined in the study: Group I comprised of 15 staff who had operated for at least ten years, while Group II consisted of 15 healthcare members who had worked in operating rooms for more than ten years. In addition to control group consisted of 15 employees who had never been exposed to anesthetic agents. The comet assay was used to detect the damage of DNA in whole blood samples. Serum malondialdehyde (MDA) values were used to assess oxidant stress, and superoxide dismutase (SOD) and glutathione peroxidase (GPX) activities were used to assess antioxidant response (SOD). **Results:** The result indicates that the mean comet variables have increased (tail DNA percentage, olive tail moment, and tail moment). In Group I, the most DNA damage was observed. There were important positive associations between years of anesthetic gas exposure and damage to DNA. In addition, in comparison to the control group, there was a significant decrease in serum GPX activity and rises in serum levels of both MDA and SOD activity in Groups I and II. **Conclusions:** According to the results, years of working as an anesthetic can damage DNA and lipid peroxidation. As a result, safe waste anesthetic gas concentration limits in operating rooms, as well as precautions for professionals who might be exposed, are important.

Keywords: Anesthetic gases; DNA damage; comet assay; oxidative stress; occupational exposure.

INTRODUCTION

The possible future negative health effects for exposures of operating room personnel (ORP) to inhalation anesthetics are of particular concern. Chronic occupational exposure of ORP to volatile anesthetics occurs mainly from waste anesthetic gases (WAGs) of patients breathing. Since halogenated anesthetics like halothane, sevoflurane, desflurane, isoflurane, and nitrous oxide (N_2O) are the most widely available anesthetics, they make up most WAGs **(NIOSH, 2007).**

It is widely acknowledged that even brief contact has a negative impact on health care staff, which manifests itself in several symptoms and signs, including Headache, nausea, dizziness, impaired judgment, and balance are some of the symptoms **(NIOSH, 2007)**. Furthermore, this exposure lasts for several years, resulting in more serious effects such as kidney and liver damage, Parkinson's disease, and neurodegenerative disorders **(Casale et al., 2014).**

Much research on impact of vaporized anesthetics on genetic materials (genotoxic and mutagenic effects) in animals, patients, and professionals have been performed **(Casale et al., 2014)**. There is a well-

established link between anesthetic exposure and the occurrence of DNA damage **(Rozgaj et al., 2009)**.

To identify genotoxic substances with a possible carcinogenicity, genetic indicators have been widely used. One of the most common procedures for detecting genotoxicity is the comet test. **(Norppa, 2004)**

Comet test is a quick, accurate, and a secure way of detecting the existence of DNA defects, which is a powerful predictor of consequences of genotoxicity on cells triggered by physical factors and chemicals, it is frequently used in human cells to assess DNA damage. **(Collins, 2004)**

The difference between ROS development and antioxidant defense is oxidative damage; these radicals are unstable molecules having unpaired electrons. Oxidant radicals are formed as a result of oxidation and known as ROS, and when they come into contact with biological molecules, a chain reaction occurs, resulting in lipid peroxidation, protein injury, and nucleic acid oxidative damage **(Ceppi et al., 2010)**.

Oxidant radicals, which cause cell damage, necrosis, and apoptosis, eventually harm tissues and organs. It has been discovered that oxidative stress and genotoxicity are related. Oxidative stress causes cellular damage due to its harmful effects on macromolecules including proteins, nucleic acids, and fatty acids **(Lee et al., 2015)**.

Even though the mechanisms of halogenated anesthetics' genotoxic and mutagenic effects aren't fully understood, oxidative damage capable of producing reactive oxygen species with direct DNA harm at any step of the cell cycle **(Chinelato and Froes., 2002)**.

The study's goal is to assess the extent of oxidative damage and DNA degradation among staff in the operation room and compare working years of exposure to these anesthetics in operating rooms to the extent of damage to DNA, using comet test to assess systemic DNA damage in blood cells, and using oxidative-antioxidant markers to assess oxidative stress.

SUBJECTS AND METHODS

The Human Research Ethics Review Board at Assiut University in Egypt gave their approval to this report. All the participants signed a written informed consent document. The research included 45 participants of both sexes, between the ages of 23 to 43 years. Thirty of them work in Egypt's Assiut University Hospital's anesthesiology department. Based on working years, members exposed to waste anesthetic gases were divided into two equal classes (15 in each). Group I was exposed to waste for less than 10years & group II was exposed to more than 10years. In addition, the control group was made up of 15 academic department employees who had not been contacted to anesthetic exhaust gases. The study excluded smokers, alcoholics and everyone who had recently been exposed to radiation.

Alkaline comet assay

In an EDTA-containing vacutainer tubes, whole blood samples were drawn and used for the comet test fairly quickly. The alkaline comet was conducted out using **Singh et al** process's **(1988)**. On frosted slides, 100 µl of 1 percent natural melting agarose was added. A total of ten milliliters of whole blood is gelled after being suspended in 2% low melting agarose. Finally, 100µl of 1% normal melting was quickly applied on top of the ice, and then another slide was added as a cover to the gel. The slides were submerged in lysis buffer once the agarose covering had dried (1 percent sodium sarcosinate, 100 mM Na_2EDTA, 2.5 M NaCl, 1 percent Triton X-100, 10 mM Tris-HCl, and 10% DMSO) at 4°C in the dark for 60 minutes.

On a horizontal gel electrophoresis apparatus, the slides were positioned (Cleaver Scientific Ltd., UK) and soaked in cold alkaline electrophoresis buffer

solution (1 mM Na_2 EDTA and 300 mM NaOH at pH 13) for 20 minutes at ambient temperature to enable DNA unfolding

Electrophoresis was carried out in a horizontal electrophoresis platform for 20 minutes at 300 mA and 25V in fresh, chilled electrophoresis buffer. Until microscopy, the slides were neutralized three times with Tris-HCl buffer (pH 7.5) for five minutes per time and stained with 50 ml (20 g/mL) ethidium-bromide. A fluorescence microscope (Olympus BX-43, Japan) fitted with a green filter was used to analyse the nuclei on the slides (3 slides per sample) at a 200-fold magnification. A digital camera was used to capture the image of the cells. The Comet Assay Software Project (CASP) was used to calculate length of tail, DNA percentage in tail, Olive tail moment and tail moment in at least 50 nuclei per slide.

Oxidant/Antioxidant Assessment

To isolate serum, blood samples were collected in clear sterile tube and used for oxidative status assays. The extent of serum malondialdehyde (MDA) was calculated using the Buege & Aust (1978) method to determine lipid peroxidation. The antioxidant assessment was performed by assessing superoxide dismutase (SOD) and glutathione peroxidase (GPX) activities in samples using the Misra & Fridovich (1972) and Beutler and Kelley (1963) procedures, respectively.

Ethics approval

The Research and Ethical Committee of Assiut University's Faculty of Medicine gave their approval to research protocol. The ethical number given is "17300580". The individuals who took part in the study gave their informed consent. The data's confidentiality was ensured.

Data analysis

For each group, the study's results were shown as a mean and standard deviation and statistical analysis was carried out using the Independent-t test, ANOVA test, and Spearman correlation tests. When p0.05 was used, the results were found significant. The software package SPSS for Windows (version 20) was utilised (SPSS Inc., Chicago, IL, USA).

RESULTS

In terms of demographic statistics, there were no notable differences across groups (age, sex, and BMI) (Table 1).

Table (1): Demographic data of the groups.

	Control group	Group I	Group II	P value
Age	32.71±3.85	32±4.11	38.79±2.52	3.51
Gender (M/F)	10/5	9/6	11/4	>0.05
BMI (kg/m^2)	24.18±0.51	22.3±8.53	26.63±1.67	0.47

P value< 0.05 is significant.

In terms of comet data, a microscopic fluorescent analysis of control subject's blood revealed intact DNA, as shown in Figure 1. (a &b). Anesthetic personnel's blood, on the other hand, showed minimally compromised cells as evidenced by increased tail length Figure 1. (c & d), statistical analysis supported these morphological observations, tail length, percentage of tail DNA and tail moment demonstrates that there was a substantial difference between the control group and group I, and the tail DNA percentage and tail moment showed a significant difference between group II and the control group. The only parameter that showed a substantial difference between groups I and II was the percentage of DNA in the tail (table 2).

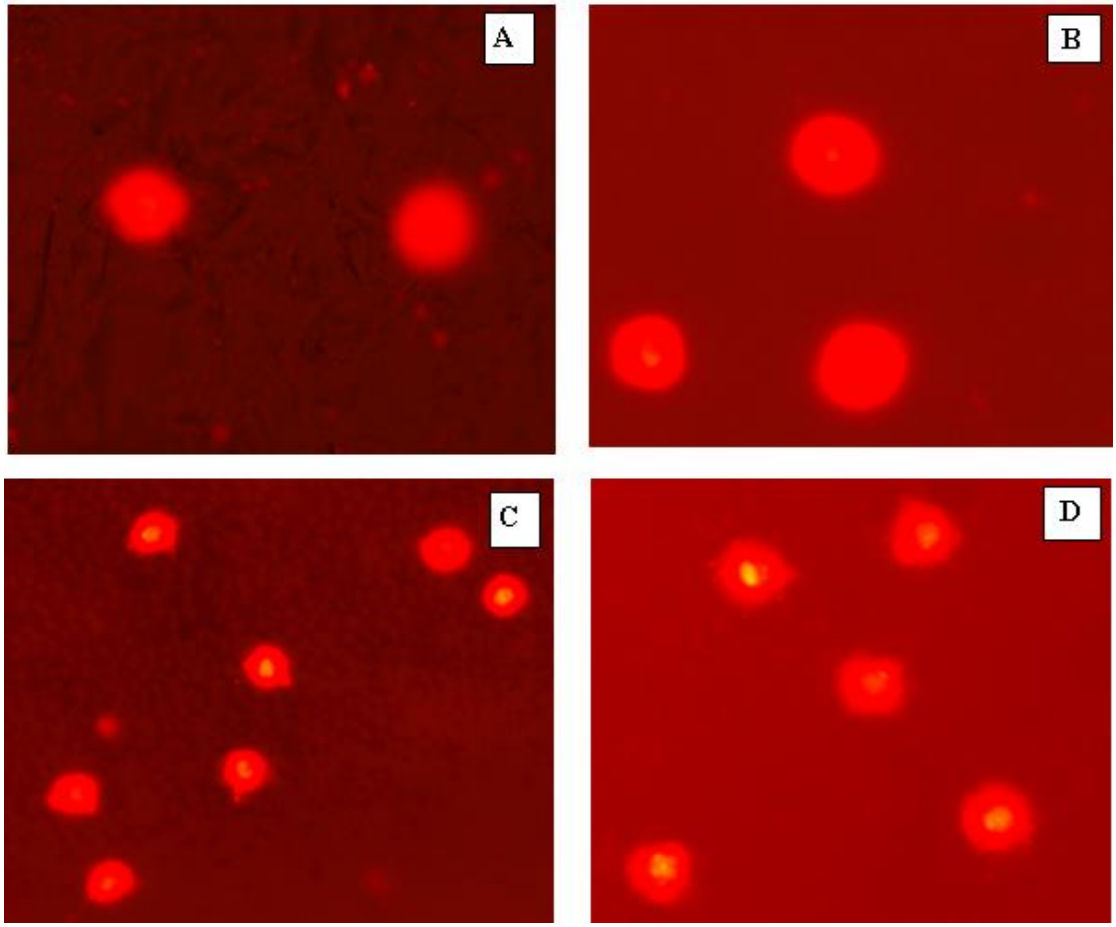

Figure (1): A &B: photomicrographs of peripheral blood of control group, showing fluorescent spheres without tail. C &, D: photomicrographs of peripheral blood lymphocytes of operating room personnel, showing minimal change in fluorescent heads with tails. (ethedium bromide stain; microscopic magnification 200).

Table (2): Comet parameters of blood cells of the three group subjects (the data are shown in mean and standard deviation).

	Control	**Group I**	**Group II**	**P-value**
Tail length (mm) Mean ± SD	3.8±0.54	3.95±0.11[a]	4.36±0.23	0.25
Percentage of DNA in the tail Mean ± SD	0.04±0.02	0.13±0.05[a]	0.17±0.02[b,c]	<0.001*
Tail moment Mean ± SD	0.016±0.014	0.015±0.01[a]	0.05±0.008[b]	0.04*
Olive tail moment Mean ± SD	0.065±0.028	0.151±0.0345	1.84±2.32	0.025*

SD: standard deviation.*Significantly different between groups (p < 0.05) as determined by Independent t test & ANOVA test.[a:] p-value <0.5% between group I, control group, [b]: p-value <0.5% between group II, control group, c : p-value <0.5% between group I, group II.

With the increase in the length of exposure to waste anesthetics gases, there was a statistically significant rise (p<0.05) in the proportion of DNA in tail, tail moment, and olive tail moment in all members of the control group relative to groups I and II. However, there was no significant statistical difference (p>0.05) between the tail length groups with the increase in years of exposure to WAGs. (See Table 2)

MDA increased significantly between controls and studied groups (I and II), as well between groups I and II, as shown in Table 3. SOD enzyme activities increased significantly between the controls and group I, while GSH enzyme activity decreased significantly between groups I & II and between groups I & II.

The effects of WAGs on oxidative injury and antioxidant condition on both classes of ORPs have been studied. The exposed groups had significantly higher levels of oxidative damage (Table 3), and MDA, a lipid peroxidation marker, was substantially greater ($p<0.001$) than the control group, group I, and group II. To overcome oxidative damage, the activeness of the antioxidant enzyme showed a significant decrease in GSH ($p<0.01$) and a significant rise in the activity of the SOD enzyme ($p<0.05$) (table 3).

Table (3): Comparison of the oxidant/antioxidant stress parameters between the groups.

	Control	Group I	Group II	P-value
MDA(µmol/L) Mean±SD	0.758±0.202	1.74±0.14[a]	3.23±0.95[b,c]	<0.001*
SOD (U/l) Mean±SD	149.82±48.68	194±24.37[a]	292.25±44.72	0.002*
GSH(U/l) Mean±SD	98.68±9.03	83.97±2.97[a]	81.1±8.96[b,c]	0.08*

MDA, malondialdehyde; SOD, Superoxide dismutase; GSH, glutathione, SD: standard deviation, *Significantly different between groups ($p < 0.05$) as determined by Independent t test & Annova test, [a]: p-value <0.5% between group I, control group, [b]: p-value <0.5% between group II, control group, c : p-value <0.5% between group I, group II.

There were significant positive associations between the duration of exposure (years) to WAGs and DNA damage in comet parameters [tail DNA percent r= 0.694, $p<0.001$, tail length r= 0.7, $p<0.001$, tail moment r= 0.324, $p<0.05$, and olive tail moment r= 0.599, $p<0.001$] shown in figures 2, 3, 4 & 5. Instead regarding to the relation between working years and oxidative damage, there was a significant positive correlation between MDA and working years (r= 0.798, $p<0.001$), SOD (r = 0.823, $p<0.001$), and a significant negative association between GPX and working years (r= -0.709, $p<0.001$) (figures 6, 7 & 8).

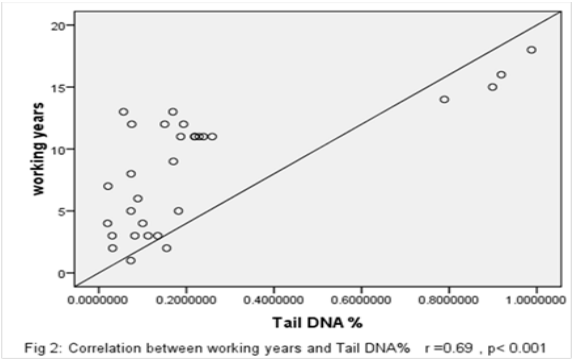

Figure (2). Represents significant positive correlation between working years and Tail DNA% r =0.69, p< 0.001

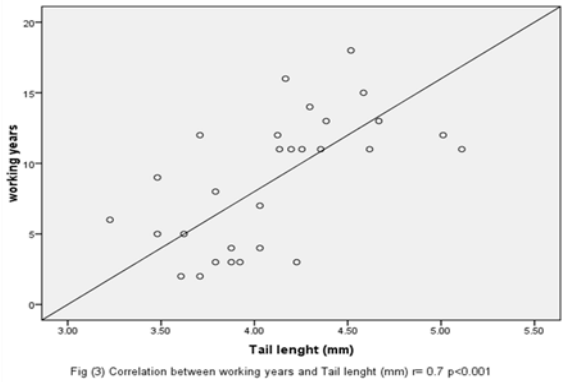

Figure (3): Represents significant positive correlation between working years and Tail length (mm) r = 0.7 $p<0.001$

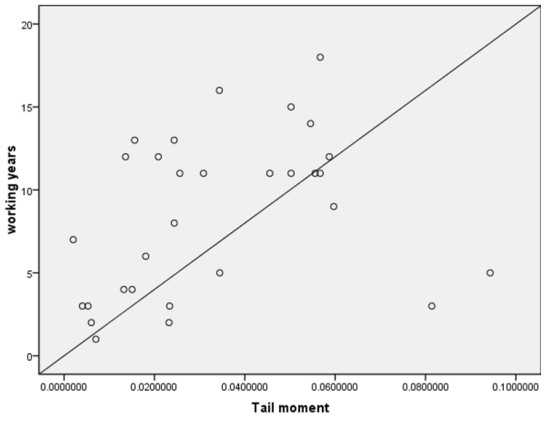

Fig 4: Correlation between working years and Tail moment r= 0.32 p<0.05

Figure (4): Represents significant positive correlation between working years and Tail moment r= 0.32 p<0.05

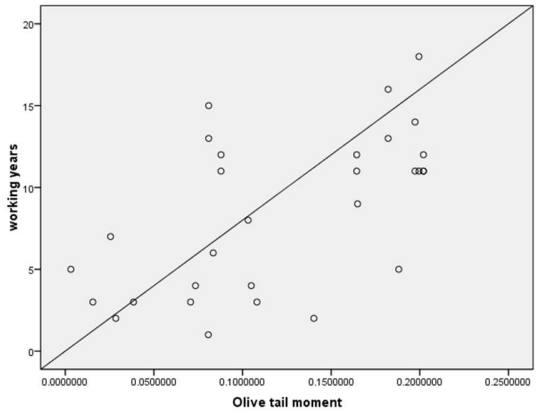

Fig 5: Correlation between working years and olive Tail moment r = 0.599, p<0.001

Figure (5): Represents significant positive correlation between working years and olive Tail moment. r = 0.599, p<0.001

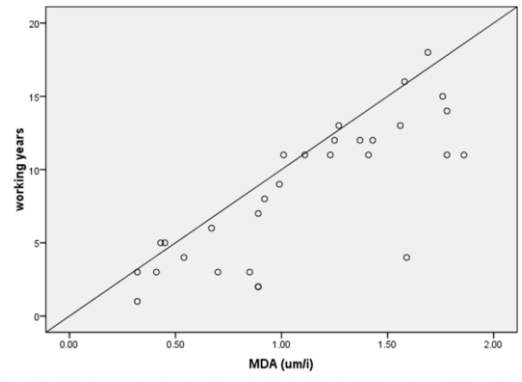

Fig 6: Correlation between duration of work (years) and malondialhyde (MDA) umol/L r = 0.798, p<0.001

Figure (6): Represents significant positive correlation between duration of work (years) and malondialhyde (MDA) umol/L r = 0.798, p<0.001

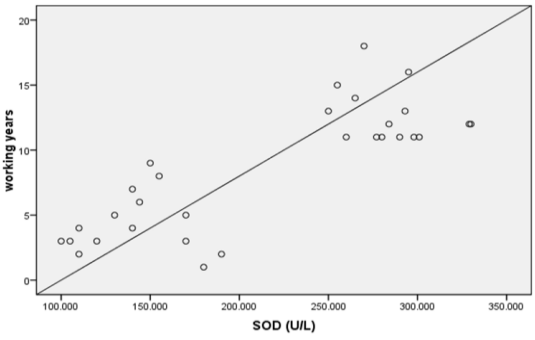

Fig 7: Correlation between working years and Superoxide dismutase (SOD) r = 0.823, p<0.001

Figure (7): Represents significant positive correlation between working years and Superoxide dismutase (SOD) r = 0.823, p<0.001.

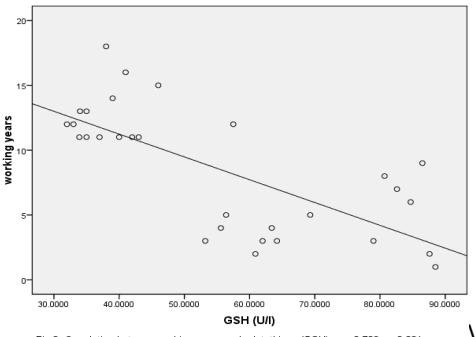

Fig 8: Correlation between working years and, glutathione (GSH) r = - 0.709, p<0.001

Figure (8): Represents significant negative correlation between working years and, glutathione (GSH) r = - 0.709, p<0.001.

DISCUSSION

The occurrence of health harm associated with the exposure to anesthetics has been the subject of debate in recent decades (**Lucio et al., 2018)**. DNA damage associated with different anesthetics has been examined using various detection methods **(Zare Sakhvidi et al., 2012).** Thus, this research was conducted to evaluate the genetic harm of contact to WAGs by using comet test, which is considered a helpful method to detect the extent of DNA breaks directly after it happened in even a single cell **(Rozgaj et al., 2009).**

The current study found a steady rise in DNA injury in lymphocytes exposed to WAGs, as measured by large increases in tail moment, olive tail moment, and the percentage of DNA in the tail, compared to controls using the comet test. These results suggest that DNA damage caused by

anesthetic exposure in the workplace is substantially higher than the permissible safe levels.

These findings were corroborated by a study of 17 physician assistants who were exposed to anesthetic gases and there was a important changes in comet parameters among the control and exposed groups **(Izdes et al., 2009)**, other experiments that used the comet test to measure the effects of aesthetics on genetic material found contradictory results. These studies attributed the discrepancy to several parameters, including the length of exposure, the conditions of exposure, the various types of anesthetics, and individual differences. **(Faust et al., 2004).**

Although these conclusions do not enable us to draw any assumptions about the probable mechanism of the genotoxicity of those anesthetic agents, it has been hypothesized that inhalational anesthetics can trigger increased oxidative stress (inadequate antioxidant capacity and excessive emission of oxidising agents), resulting in reactive products that cause oxidative DNA harm **(Baysal et al., 2009).**

In the current study, anesthetics staff showed oxidative injury to DNA, as well as a disturbance of the pro-oxidant/antioxidant equilibrium, with inadequate antioxidant capacity and emission of excessive oxygen radicals. The disruption of the oxidant/antioxidant equilibrium is among the causes of oxidative stress to cell components including DNA **(Baysal et al., 2009).**

Furthermore, different theories about the mechanisms of anesthetic-induced DNA damage have been proposed. For example, some drugs can harm DNA directly or via their active metabolites **(Jaloszynski et al., 1999)**, or function as radiomimetic drugs, causing damage to cell cycle phases (S-independent compound) **(Chinelato, 2002)**, moreover, some anaesthetics promote methionine synthase cyanocobalamin (vitamin B12) reduction, which is followed by the production of superoxide and hydroxyl radicals, as well as the suppression of enzymes that cause homocysteine remethylation to methionine (**Drummond and Matthews, 1994).** According to this study, the exposed groups had a large increase in MDA levels in comparison to the control group. The antioxidant enzyme activity of GPX was found to be significantly reduced in anesthetics that were chronically exposed to WAGs in this study. Conversely, SOD enzyme activity is significantly increased in groups exposed to anesthetic agents, indicating increased oxidative damage induced by anesthetic exposure.

According to **Akbar et al. (2005)**, anesthetic gases increase peroxidation of lipids and oxygen radicals, which have increased blood oxidative damage in exposed individuals at low doses. This suggests that oxygen radicals have increased and can destroy cells, potentially causing serious harm to multiple organs in the coming years.

The findings of this study revealed a correlation between health professional's exposure to WAG and the induction of genotoxicity, as well as a correlation between dose and genotoxic effects. Extended exposure to anesthetics has been correlated to carcinogenesis, mutagenesis, teratogenesis, and reduced fertility **(Chandrasekhar et al., 2006)**. Several reviews found positive associations between occupational disease occurrence and exposure to hazardous and gaseous chemicals, contradicting the research findings **(Peric et al., 1991)**.

Despite validated scavenging mechanisms, chronic exposure to low concentrations of anesthetic agents has been suggested to be harmful to operating room workers in several studies. The findings revealed strong positive associations between longer periods of WAG exposure, comet parameters, and lipid peroxidation, while a significant negative correlation was found between years of exposure to anesthetics and GPX activity.

CONCLUSIONS

In this study, there was a positive correlation between chronic occupational WAG exposure and DNA damage and oxidative stress. Since this work was performed on a small number of people, the results could be skewed. The research should include a greater community of doctors and nurses who are attached to anesthetics. In operating rooms, anesthetic levels must still be controlled. Human interference by education and procedures to ensure that anesthetic techniques are used to reduce air emissions in the area, along with environmental action through the introduction of successful OR scavenging technologies.

List of Abbreviations

MDA: Malondialdehyde, **OR:** Operating Room, **GPX:** Glutathione peroxidase, **WAGs:** waste anesthetic gases, **SOD:** Superoxide dismutase, **GSH:** Glutathione, **EDTA:** Ethylenediaminetetraacetic acid, **ORPs:** Operating Room Personal.

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السمية الجينية و الجهد التأكسدي بين أطباء التخديربإستخدام مقياس المذنب القلوي للحمض النووي

إيمان صلاح الدين شلتوت[1] ، ضحا يحيي [2]، نها إسماعيل إبراهيم[1]

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قد يؤدي التعرض لمواد التخدير عن طريق الاستنشاق على المدى الطويل إلى مخاطر صحية لا يمكن استبعادها تمامًا. الهدف من الدراسة: هو معرفة كيف تؤثر غازات التخدير إلي تلف الحمض النووي والضغط التأكسدي. طرق البحث: انضمت إلى الدراسة مجموعتان من العاملين في مجال الرعاية الصحية من قسم التخدير: المجموعة الأولى تتألف من 15 فرد عملوا لمدة عشر سنوات على الأقل ، بينما تألفت المجموعة الثانية من 15 فرد من أفراد الرعاية الصحية الذين عملوا في غرف العمليات لأكثر من عشر سنوات. بالإضافة إلى المجموعة الضابطة المكونة من 15 فرد لم يتعرضوا أبدًا لادوية التخدير. تم استخدام اختبار المذنب للكشف عن تلف الحمض النووي في عينات الدم الكاملة ،تم قياس المالونديالدهيد كعلامة عن اكسدة الدهون وكذلك قياس مجموع الانزيمات الكلية المضادة للأكسدة لتقدير الدورالمحتمل للجهد التأكسدي. النتائج: تشير النتيجة إلى زيادة متوسط نسبة تذيل الحمض النووي. في المجموعة الأولى ، لوحظ أكبر ضرر للحمض النووي. الطلاء بوجود زيادة ذو دلالة إحصائية بين سنوات التعرض لغازات التخدير وتلف الحمض النووي. بالإضافة إلى ذلك ، بالمقارنة مع المجموعة الثالثة و انخفاض ذو دلالة إحصائية في مستوي إنزيمات مضادات الأكسدة وجود زيادة ذو دلالة إحصائية في مستوي المالونديالدهيد و السوبر اوكسيد الدسميوتاز في المجموعتين الأولى والثانية. الاستنتاجات خلصت نتائج الدراسة إلي إن الضرر بالحمض النووي وأكسدة الدهون يزداد مع سنوات التعرض للمواد المخدرة ، ولذلك وجب اتباع الاحتياطات للمهنيين الذين قد يتعرضون للمواد المخدرة و تحديد تركيز غاز التخدير الآمن في غرف العمليات.