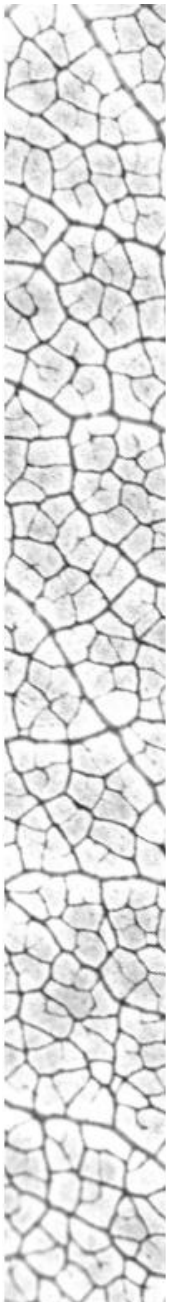

EurAsian Journal of BioSciences
Eurasia J Biosci 14, 2975-2981 (2020)

Effects of halothane on the peripheral lymphocytes and buccal mucosa exfoliated cells in operation room workers

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Abstract

Background: Halothane is one of commonly inhalation anesthetic use in Iraq and the rest of the countries. Anesthetic is substance which reduce sensitivity to pain or is drug used to induce anesthesia that lead to temporary loss sensa-tion or awareness consciousness and which block only sensation of pain stimuli

Aim of study : This study was requested by Iraqi ministry of health to focus on the side effects of halothane on the patients and health,a study was perform-ed to evaluate the effects of the halothane anesthetic waste to induced sister chromatid exchange (SCE) and micronuclei (MN) in peripheral lymphocytes and buccal cells respectively in operation room workers (ORWs).

Material and Methods : SCE and MN,a sensitive measure of genotoxicity were counted SCE in peripheral blood lymphocytes and MN in buccal mucosa cells for 40 ORWs exposure to halothane gases waste and 20 healthy person as con-trol who had never works in operation room.

Results : All of the ORWs exposure to halothane gases waste, have cells with SCE and MN frequencies, in analysis, ORWs was significantly increased SCE in ≥ 10 year and ≤ 5 year compared with control group with mean±SD (11.31± 0.9 and 7.67±0.33)respectively vs mean±SD (5.55±1.6), P< 0.001).fothermore, a significantly increased higher MN frequency with mean± SD (7.53±0.78 and 5.02±0.53) in ORWs with ≥ 10 year and ≤ 5 year respectively comparison with their control group with mean±SD (2.40±0.40),(p<0.001).Also non-significant differences in SCE and MN frequencies according to the gender.

Conclusion : The chromosome damage and genotoxicity are related to the ORWs daily exposure to halothane waste and may be increased by accumula-tive with duration of exposure to free radical and reactive oxygen species lead to damage in their cells of peripheral lymphocytes and buccal cells over time of work.

Keywords: Hallothane, Sister Chromatid Exchange, Micronuclei, Anesthetic

AL-Quraishe FA, AL-yasari AM (2020) Effects of halothane on the peripheral lymphocytes and buccal mucosa exfoliated cells in operation room workers. Eurasia J Biosci 14: 2975-2981.

INTRODUCTION

Inhalational anesthetics are the most commonly used agents for minanten-ance of general anesthesia in animal and human (Cattano, et al 2008). The popularity of these drugs to establish anesthesia is based on arrange of advantages, such as eassy of administration predictability of their effects and low coast (Kleinsasser, et al(2001) The anesthe-tics agents that used the present time are halothane, enflurane, isoflurane, sevoflurane and desflurane (Della Rocca, et al. 2001) . Halothane is one of commonly inhalation anesthetic used (Topal, Gal, Lio & Gorgul 2003). The chemical structure of halothane 2- bromo 2-chloro 1, 1, 1-trifluoroethane (McLain, Sipes & Brown 1979) About 80% of inhaled halothane is taken by body is eli-minated unchanged via lungs, and about 20% of halothane not eliminated in exhaled gas, is metabolized in liver to principal metabolite, trifluoro acetic acid(CF3CooH) (Rosnak, Halevy & Orda 1989) –(Mikhail, Murray, Morgan 2006 Trifluoro acetic acid is a stable end product of halothane metabolism, which is most eliminated in the urine (Sharifi & Vesal 2005) Many reports of heap-toicosis associated with halothane administration has been published (Rodes & Brugueca 2001). The oxidative metabolism of halothane produce a trifluoro acetic acid bind with hepatocytes and act as hapten, than bind with liver proteins and induces immune response in the liver, resulting of inflammatory response in the liver is susceptible individuals (Harper 2004). Degenerative effects of halothane on liver, kidney and brain have been reported (Eger 2004). Many studies have explained the relation between anesthesia and

Received: October 2019
Accepted: March 2020
Printed: September 2020

generation of free radical and the role of free radicals in emergency of disease, free radicals act on oxidations of macromolecules in the development of many diseases (Gupta & Chair 2006). Al though toxicity potential of haloth-ane on the biological systems needs more studies to determination the effects the halothane on the induced the genotoxicity and DNA damage. In these study we are examination the effects of halothane anesthetic waste gases to genotoxicity and DNA damage on Sister Chromatid Exchanges (SCEs) in the peripheral blood lymphocytes and Micronuclei (MN) in the exfoliated buccal mucosa cells of Operation Room Workers (ORWs) respectively .

Sister chromatid exchange, is a popular method in genetic toxicology and hu-man population cytogenetic monitoring (Lazutka 1995). SCEs phenomenon is widely used as reliable and sensitive indicator of chromosome (DNA) instability (Konta 2003). Since SCEs are consider to be sensitive indicators of genetic material effects after exposure to mutagenic and carcinogenic agents (Nakanishi, Schneider 1997. Solomon & Bobrow 1975)), the SCE patterns can reveal a general genome instability, variation in (DNA) repair mechanisms or detoxicity enzymes have been implicated as causing genetic susceptibility asso-ciated with cancer (Gebhart 1981. Konta 2003). SCE in peripheral blood lymphocytes, has been widely used assess exposure to mutagenic and carcinogens (Wolf 1991. Therman, Susman 1993).

Molecular epidemiology research focuses on the biomarkers of exposure (e.g cytogenetic endpoints, chromosome aberration (CA) micronuclei (MN), SCE and apoptosis) (Tolbert,Shy & Allen 1992 – Stich, San & Rosin 1983 . Fenech, Crott 2002)) . MN assay has been applied to evaluate chromosome damage for biological monitoring of human population exposed to variety of mutagenic and carcinogenic agents (Martinez 2005 – Pastors, Creus, Xamena, Siffe & Marcos 2002 - Pastors, et al. (2001).

The objective of the current study was to investigate the spontaneous genetics damage in exfoliated cells of buccal mucosa from ORWs exposure to halothane anesthetic waste gases agents.

MATERIAL AND METHODS

Sample Collection

Samples: 40 health (anesthetists, nurses, and technicians) (22 males and 18 females) operation room workers in Ad-Diwaniyah teaching hospital exposure to waste of halothane gases were examined in this study. In addition 20 health (12 males and 8 females) available hospital staff works to other wards, with – out the history of working in operation room were control group. The ORWs and control groups individuals were aged (Pastors, et al. (2001). Shekell, Morton, Maglione, et al. (2004). year's. A written consent was taken from each individual and the samples were taken from. The period of study was extended for 6 months.

This study was approved by hospital ethical committee approved the human study.

Sister Chromatid Exchanges Estimation: Blood samples from each individual a peripheral blood samples was collected by venipuncture in heparinized tubes and were cultured within less than one hour of sampling (Rantanen, Grenman, Kulmala & Grenman R 1994– Khabour, Soudah, Aaysa 2013; Olowa, & Olowa, 2015). This study is a laboratory investigation that individual genotoxicity assessment effects of halothane anesthetic waste in human lymphocytes obtained from ORWs using SCES assay.

Blood culture was done by adding (1 ml) of fresh whole blood to (9 ml) of complete karyotyping media (Gibco – Invitrogen, Paisley, UK). Cultures were incubated in the dark at 37 C^{o} for (72 hours) in 5% Co_2 atmosphere with appro-priate humidity (Alsatari, Azab, Khabour, et al. (2012). Three hours before harvesting, Colchicine (0.1 µg / ml) was added to arrest the cells in the metaphases. Cultured cell were harvesting and fixed using methanol / acetic acid procedure as previously described (Sadiq, et al. 2000) – Perry,Wolf 1974)). Slides were prepared and stained by Fluorescence–plus- Giemsa technique (Therman,Susman 1993) – Perry,Wolf 1974)), SCEs from one hundred well spread second metaphase with good differentiate were scored for SCEs analysis for each individual (Li, Maglione,Tu, et al.(2006).

Micronuclei assay: The samples of exfoliated oral mucosa cells were collected from ORWs and control group. The participants were also asked to rinse the oral cavity for 1minute with 10ml of sterilized distilled water and the exfoliated cells of buccal mucosa were obtained by a light and gently pressure was applied, while scraping the buccal mucosa with cytobrush moistened with buffer (Chen, Arjomandi, Qin & et al(2006 . speit & Schmid 2006. Casartelli, Bonatti, De Ferrari & et al. (2000). for each individual, cytobrush used to collected buccal cells was shaken in centrifuge tube containing saline solution (Hank's basic or other buffer solution) to release the cells and the tube was then centrifuged to wash the cells in the buffer solution twice. The supernatant was discarded and pellets were re-suspended in 0.75M Kcl and fixed by cold methanol-glacial acid mix (3:1)then the tubes stored at room temperature until investigation of MN. Slides were prepared by adding one drop of fixed cells solution onto center of clean glass slides was dried in air . Staining was carried out with 2% giemsa sol-ution for a period of 10 minutes, after wards, the glass slide was rinsed with distilled water and dried with air.

The MN evaluation screened for cells abnormalities, addition to counting MN is also investigated under oil immersion lens (1000X), followed by phase contrast microscopy for counting of MN according to established

Table 1. Comparison mean ±SD SCE frequency in second division metaphase in ORWs

Subjects	Duration of Exposure to Halothane	Sister Chromatids Exchanges		
		No. of cells scored	SCE / Cell (Mean±SD)	SCE Rang
ORWs No.40	≥10 year No. 18	1000	11.31±0.9**	(8 – 14)
	≤5 year No. 22	1000	7.67±0.33	(8 – 14)
Control No. 20	0	1000	5.55±1.6	(3 – 6)

Table 2. Comparison SCE frequency in second division metaphase in ORWs and control groups according to the gander. Significant P < 0.001 **

Subjects	Duration for Exposure to Halothane	Sister Chromatid Exchanges		
		No. of cells scored	SCE/Cell (Mean± SD)	SCE Rang
ORWs Males No.22	≥10 year No.12	1000	12.2±1.9**	(8 – 16)
	≤5 year No.10	1000	8.75±0.33**	(6 – 10)
ORWs Females No.18	≥10 year No.8	1000	10.42±1.9**	(8 – 14)
	≤5 year No.10	1000	6.61±24	(6 –10)
Control Males No. 12	0	1000	5.91±0.31	(3 – 6)
Control Female No.8	0	1000	5.22±1.0	(3 – 6)

Table 3. Micronuclei cell mean ±SD frequency in buccal mucosa exfoliated cells of ORWs and control groups

Subjects	Duration of Exposure to Halothane	MN – Rang	MN (%) (Mean±SD/1000cells)	Comparison	PValue
ORWs No.=40	≥10 year No.=18	1 – 18	7.53 ± 0.75 *	≥10 year versa Control	P<0.001
	≤ 5 year No.=22	1 – 18	6.22 ± 2.47	≥10 year versa ≤ 5 year	
Control No.=20	0	0 – 6	3.17 ± 0.40*	≤5 year versa Control	P<0.001

Significant P <0.001 *

methods (speit & Schmid 2006. Titenko - Holland et al. 1998. Bonassi, Neir,Puntoni 2001). At last 1000 intact epithelial cells/ individual were scored to achieve the average percent of MN cells. Statistical analysis was done by SPSS version 15, comparison by (ANOVA-LSD) and correlation by Spearman correlation.

RESULTS

The cultures of human lymphocytes of OWRs as shown in **Table 1**, halothane anesthetic waste gases significantly increased the frequency of SCEs in ORWs compared with control group (P< 0.001)these increased proportional to duration of exposure to halothane waste ≥10 year and ≤ 5 year with mean ± SD (11.31±0.9) and (7.67±0.33) respectively in comparison with their control group with mean ±SD (5.55±1.6).

As shown in results, the SCEs frequencies in second division metaphase in ORWs exposure to halothane anesthetic waste significant they was increase (P < 0.001) mean±SD (11.31±0.9) with increased duration of exposure ≥ 10 year ORWs group.

The study of correlation of SCEs in ORWs exposure to halothane waste reveal non – significant differences between frequency of SCEs in males and females (P= 0.757, r = 0.040). In comparing cells obtained from ORWs and control groups, non – significant differences in SCE mean occurrence was observed in relation to gender (**Table 2**). Also, result shown non statistically significant differences between SCEs frequencies between ≥10 year and ≤ 5 year with (P = 0.001).

Micronuclei analysis, results showed (Table 3) the MN frequency was significantly higher (P < 0.001) in buccal mucosa cells obtained from ORWs exposure to halothane waste proportional with duration of exposure ≥ 10 year and ≤ 5 year with mean ±SD (7.53±0.78) and (5.02±0.53) respectively compare with the control group with mean ± SD (2.4±0.40). The correlation present study report (**Table 4**) no statistically significant differences in MN frequencies were compared according to gender in ORWs (≥ 10 year and ≤ 5 year) (P =0.001). However, all ORWs had a significant frequency increase MN mean ±SD between sample at≥ 10 year and ≤ 5 year when compared with control group.

DISCUSSION

In this study, the adverse effects the halothane gases waste induced genotoxicity in ORWs was examined using SCEs and MN assessments.

The results showed that the genotoxicity of halothane anesthetic waste was significantly increased SCEs and MN by increased the time of exposure ORWs to halothane gases waste.

Halothane inhaled anesthetics with high – fat solubility is used for a prolonged period (Eger (2004). . Some adverse effects observed after the use of various anesthetics (deflurane, isoflurane and halothane) could be explained by the disorder of cellular ion homeostasis

Table 4. Comparison mean± SD micronuclei cell frequency in buccal mucosa exfoliated cells of ORWs and control groups according to gender

Subjects	Duration Exposure to Halothane	MN Range	MN(%) (Mean±SD/1000Cells)	Comparison	P Value
ORWs Male No.=22	≥10 year No.=12	1 – 18	7.12± 2.52	≥10 male versa ≥10female	
	≤5 year No.=10	1 – 18	6.64± 1.81	≤5 male versa ≤5female	
ORWs female No.=18	≥10 year No.=8	1 – 18	6.56± 2.36 *	≤10 female versa female control	P≤0.001
	≤5 year No.=10	1 – 18	6.45±2.52 *	≤5 female versa female control	P≤0.001
Control No.20	Male No.=12	0.6	3.65±2.04*	Control male versa≥10 male	P≤0.001
	Female No.= 8	0.6	3.60± 2.26*	control female versa ≥10 female	

Significant P <0.001 *

and lung clearance (Smuszkiewicz et al. 2006).Our results indicate the halothane,induces SCE in peripheral blood lymphocytes and MN formation in exfoliated oral mucosa ofORWs exposure to waste of halothane anesthetic, which is ultimately not along–lasting eff-cts in otherwise healthy individuals.

The peripheral lymphocytes and buccal mucosa of ORWs are exposed to high level of anesthetic gases during the anesthetic and at the end of anesthesia by exhalation of the gases in operation room environment from patient and inhalation by workers.

Damage to genetic materials can be cytologically observed as chromosomes aberration and sister chromatid exchange, suggests exposure to genotoxicity and possibly carcinogens (Pongsavee 2003).Many studies have explained the relation between anesth-esia and generation of free radicals (39),oxygen is one the most important types of free radical common in the biological system called free oxygen radical (FOR) (Pongsavee 2003), and reactive oxygen species (ROS) that are generated in the body attack the cells causing significant effects on them and have a role in the destroying their compone – nts of macromolecules include DNA(Shekell, Morton, Maglione, et al. (2004).The increase in the production of free radical lead to occurrence the case of oxidative stress, which has role in many disease(Jordan et al. 2014. Padill-Raygoza, et al. 2019).

MN assay showed of DNA damage caused by halothane in buccal mucosa of ORWs according to duration of exposure to halothane anesthetic wastes gases. New experiments point out the genotoxicity effect of halothane anesthetics and demonstr-ate that lower halothane concentrations could provoke DN damage, although the anesthetic does not interact directly with DNA, it has been found irreversible impair ment of cell genome at 5 m^M concentration (Topouzova et al. 2007).

Halothane is metabolized by one of two pathway, it responsible for the hepatotox-ic effect of halothane, may bind to cellular macromolecules and react with free amino group of proteins on hepatocytes to produce more toxic metabolites hence the macr- omolecules may lose their physiological functions (Spracklin, Thummel & Kharssch 1996). The intermediary metabolites produced during transformation of volatile anesthetics are held activities of liver enzymes . They may causes cellular damage by covalent binding to cellular compon-ents such as enzymes, nucleic acids and proteins or by another mechanism.Damage cellular components may play an important role in liver cells death (Teppema et al. 2002).

The major suggested mechanism by which halothane exerts its genotoxicty effects is by its ability to induce oxidative stress inside cells, halothane has been show to generate oxygen free radical such as hydrogen peroxide, superoxide anions, hydro- xyl radicals and nitric oxide causes cellular DNA damage (Khabour, Alzoubi, Mfady & et al. (2014).

Genotoxicity of halothane in human lymphocytes and buccal oral mucosa assess with previous literature and supports the role of oxidative stress in DNA damage ind-uced by halothane.

Oral buccal mucosa and nasal cells are first line protactive cells of the digestive and respiratory systems respectively,certain irritant including volatile anesthetics are, known to interfere with first line protective cells of respiratory and digestive systems, they show changes that are detected using microscopy (Tian, Ma,Feng, Xia, et al.(2001)

The analysis of MN in exfoliated buccal and nasal cells is a sensitive method for monitoring genetic damage in human population, especially because of its non- invasive application nature (Kesimci,coskun,Ugur, et al(2017) . The MNs are extra nuclear DNA-containing bodies that are formed because of chromosomal breakage (clastogenicity) and/or chromos-ome loss,MN they can be microscopically evaluated (Farhadi, Jolehar & Safapour 2018 . Shashikals, Indira, Manjunath, et al. 2015).

CONCLUSIONS

Our results indicated the genotoxicity and clastogenicity potential of halothane on ORWs according to chronic exposure to waste anesthetic gases, which were detected in peripheral lymphocytes and buccal exfoliated cells of ORWs. This was probably

owing to may be accumulating free radical and reactive oxygen species, damage in their cells overtime.

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