



Effects of Desflurane exposure and Laparotomy on genomic biomarkers and hepatic histopathology in an experimentally induced liver injury model: A pilot study

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Abstract

Background: Halogenated anesthetics are known to cause hepatotoxicity that can be evaluated by the emerging genomic biomarkers, that is, miRNA-122 and miRNA-192.

Methods: Thirty Wister rats were distributed randomly in five equal groups; Control Group (group C); Desflurane Group (group D); Hypoxia Group (group H); Hypoxia + Desflurane Group (group HD); and Hypoxia + Desflurane + Laparotomy Group (group HDL). Animals in the Groups D, HD, HDL were exposed to 6% desflurane. Rats were exposed to hypoxic mixture in Groups H, HD, and HDL. Animals in Group HDL were additionally subjected to laparotomy with liver palpation. After 24 hours, plasma ALT, AST, GGT, blood and tissue miR-122, and miR-192 gene expression and histopathological examination of liver biopsies were performed.

Results: Plasma and tissue miR122 and miR192 showed significant increase ascendingly in H, D, HD, HDL groups (p value <0.05), significant elevation of ALT, AST, GGT in HD and HDL groups (p value <0.05). Hepatic histopathological findings revealed 33.3% inflammatory infiltration in group D, 50% in group H, 66.6% in HD and HDL groups. 16.7% apoptosis was detected in D & H groups and 33.3% in HD and HDL groups, while none showed necrosis.

Conclusion: This experimental study concluded that each desflurane and hypoxia has deleterious effect on hepatic integrity, which increased by their combination and aggravated by surgical influence as verified by miR-122 and miR-192, as well as histopathological examination. Assessment of hepatotoxic effects of other various anesthetics and different surgical approaches in experimental study using hepatic genomic biomarkers is recommended.

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1. Introduction

Halogenated inhalational anesthetics are known to cause hepatotoxicity, being the highest with halothane [1] and to a lesser extent with sevoflurane and desflurane [2]. Intermediate metabolites of these agents involving cytochrome P-450 2E1 (CYP 2E1) are the main cause of immuno-allergic reaction and its severity correlates with the extent of their metabolism in the liver [3,4].

Drug-induced liver injury (DILI) can be confirmed through histopathological examination of liver biopsy [5]. Since biopsies are invasive and not routinely performed, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyl transferase (GGT) are currently the standard DILI biomarkers in clinical practice but still lack sensitivity and specificity [6].

Micro-ribonucleic acids (miRNAs) have recently been recognized as genomic biomarkers [7]. The human genome has been predicted to encode over 2000 miRNAs [8]. Due to its stability and minimal invasive pattern, the detection of miRNA in body fluids as blood and urine gives them a remarkable benefit as non-invasive prognostic and diagnostic biomarkers [9–11]. They can be quantified using quantitative real-time PCR (polymerase chain reaction), next-generation sequencing technologies and microarray [12]. Circulating serum miR-122 and miR-192 are liver-specific and are proposed as potentially sensitive and specific markers of acute and chronic liver injury [13,14].

Both anesthesia and surgical stress might cause independent miRNA expression changes during experimental surgery through inhalation anesthesia and/or pain medication [15]. A temporary hepatic

pedicle occlusion during extensive liver surgery in patients with advanced liver disease leads to hypoxia and triggers a process of ischemic reperfusion injury to the liver. Inhalational anesthetics have been involved in the pathophysiology of the ischemic injury and an optimization should be investigated to minimize such effects [16].

Up to our knowledge, there is no previous study that assessed the safety of desflurane by the genomic biomarkers for early detection of any expected DILI. The objective of the present work is to evaluate the effect of anesthesia (desflurane) and surgery (laparotomy) on genomic biomarkers (miR-122 and -192 as explicit hepatic biomarkers) and hepatic histopathology in an experimentally designed hepatic injury model in rats.

The hypothesis of this study is the detection of specific hepatic genomic biomarkers and their correlation with histopathological examination of liver biopsy could give us an early warning of an impending hepatotoxic reaction to potentially toxic inhalation anesthetic and a stressful surgical intervention (Sham laparotomy) [17].

Primary outcome was the measurement of miR-122 and secondary outcomes were the assessment of miR-192, liver transferase enzymes, hepatic histopathology when exposed to anesthesia and surgery.

2. Materials and methods

This study was attained in the Experimental Laboratory Unit at Theodor Bilharz Research Institute after approval of the Ethics Committee (Federal Wide Assurance Number: FWA00010609). This prospective study was registered at Animal Research Registry Identifier ID: F1J6Y3EC on 19/12/2019.

2.1. Study protocol

Thirty male Wistar rats 3–6 months age, healthy, and weighing 150–200 grams were randomly allocated to five groups 6 rats in each group, as defined: Control Group (group C); Hypoxia Group (group H); Desflurane Group (group D); Hypoxia + Desflurane Group (group HD); and Hypoxia + Desflurane + Laparotomy Group (group HDL).

All rats, except those in Groups C and D, were given phenobarbital 0.1% that supplemented to their water for five days before the start of the experiment in a concentration of 1 mg/mL, minimally 15 mg/day per rat. Phenobarbital was administered to activate the P-450 enzyme and was withdrawn one day prior to the experiment. Each rat in the Groups D, HD, HDL was put in polypropylene cage and linked to an anesthesia machine with vaporizer to deliver 6% desflurane during a 2-hour period. The control and hypoxia groups did not receive desflurane anesthesia.

Rats received a hypoxic mixture in the form of 86% nitrogen and 14% oxygen via the anesthesia machine in Group H, HD, and HDL for 2 hours. Group D received 30% O₂ in air. The animals in the Group HDL were exposed within 2 hours to hypoxia, desflurane anesthesia, laparotomy, and hepatic manual palpation. Rats of the control group did not receive hypoxic mixture nor anesthesia. By the end of the technique, all animals took their food and water as desired.

2.2. Measurement tools

2.2.1. Material gathering and mercy killing

Rats of the control as other groups underwent collection of material and sacrifice one day after the end of procedure. The blood and liver tissue samples were aseptically collected as follows:

- (1) **Ethylenediaminetetraacetic acid (EDTA) vacutainer:** about 1 ml for the detection of **miR-122** and **-192** via quantitative real-time PCR technique after immediate RNA extraction from blood and liver tissue samples.

(a) RNA Extraction

- Total RNA extraction from whole blood was performed according to TriRNA Pure kit (version 03.28.17), Cat. No: GZX050, GZXD050 (<https://www.geneaid.com>).
- Total RNA extraction from rat liver tissue using FavorPrep tissue total RNA kit (version 0515, 2018), cat. No: FATRK000 (<https://www.favorgen.com>).

(b) Gene Expression Detection:

- Gene expression detections; was performed using HERA SYBR Green qRT-PCR kit (version 08hp421191), Cat. No: WF10303001 (<https://www.willowfort.co.uk>).

The reaction wells were loaded into the Light Cycler 480° instrument II Roche, Switzerland and started the quantitative real-time PCR program.

- (c) Analysis of results: Analysis of the results using the SYBR Green I Filter Combination (465–510), which depends on HERA SYBR® Green quantitative real-time PCR Master, comparative CT approaches were used for data analysis. The house-keeping gene U6 used as an endogenous reference to standardize probable difference in value and quantity of miR-122 and miR-192. Gene expression was evaluated virtual to the control specimens that were considered as calibrator through the formula $2^{-\Delta\Delta CT}$. The obtained

results were represented as fold change of the miR-122 and miR-192 target gene expression (<https://www.biostars.org/>).

(2) Serum vacutainer: 0.5 ml for biochemical assay of liver transferase enzymes (**ALT, AST and GGT**). After blood clotting, centrifuging the tubes at a rate of 3000 rpm was done for ten minutes then the supernatant serum was separated by careful pipetting into microcentrifuge tubes and kept in -80°C ultra-low Freezer (Nuair, USA) until time of assay. Semi-automated colorimetric assessment of liver transferases was performed strictly following the manufacturer's instructions. The results were obtained through measuring the test samples using the Photometer 5010, ROBERT RIELE®, Germany.

- (a) Alanine aminotransferase: Using ALT kit Cat No AL146 Randox Laboratories Ltd. Great Britain with normal reference range 4.25–666 U/l.
- (b) Aspartate aminotransferase: Using AST kit Cat No AS101 Randox Laboratories, Great Britain with normal reference range 4.42–657 U/l.
- (c) Gamma-glutamyl transferase: Using GGT assay kit Cat No GT8146 Randox Laboratories, Great Britain with normal reference range 3.9–1285 U/l.

(3) Histopathologic study

Liver biopsy sections were preserved in paraformaldehyde until processed, then were assessed histopathologically for the grade of inflammation, apoptosis, and necrosis. Five μm thick serial sections from formalin fixed, paraffin embedded blocks of hepatic tissue samples were stained with hematoxylin and eosin (H&E) stain.

2.2.2. Statistical analyses

No previous research was found to evaluate the impact of anesthesia and surgery on hepatic genomic biomarkers, so this study was considered as a pilot study. Thirty male Wistar rats were used and randomly allocated to 5 groups of six rats in each group.

The data were processed via a statistical package for social sciences (SPSS) version 26.0 for Windows (IBM, Chicago, IL). Continuous normally distributed variables were expressed as mean \pm standard error (SE) through a 95% confidence interval and applying the frequencies and percentage for categorical variables. Student's t-test was performed when comparing the means between groups of normally distributed variables. The results of histopathological examination of rat liver tissue obtained from this experimental study were expressed as frequency and percent. The Fisher's exact test or χ^2 test were applied to detect the distribution of the categorical variables among groups. Result was considered significant when P value $<$ 0.05. Finally, Spearman's rank correlation coefficient (r) was performed.

3. Results

Regarding the gene expression of miR-122, and -192, the obtained results revealed that plasma versus liver tissue were comparable in all groups. They were constantly upregulated in H, D, DH, and DHL groups in ascending order until the peak point with the HDL group (Table 1).

The mean values of AST, ALT, and GGT levels were significantly ($p < 0.001$) elevated in HDL and HD groups compared to C, D, and H groups. Also, the HDL group revealed a significant ($p < 0.05$) increase in comparison to HD group. On the other hand, the H and D groups showed no statistical difference compared to C group nor between each other (Table 2),

Concerning histopathological studies, control specimens were found to be free from any hepatic lesion. The incidence of inflammation grades and apoptosis in the treated groups was significantly higher in each treated group in comparison to the control group. In addition, there was significantly higher level in HD and HDL when compared to H and D groups being higher in HDL group (Table 3, Figure. 1, 2). On the other hand, there was no incidence of necrosis in all groups.

The correlation study showed that there is a significant positive correlation between miR-122 and -192 expression in plasma and their expression in liver tissue. Likewise, they were positively correlated to the liver function tests AST, ALT, and GGT as well as

Table 1. Mean values of miR-122 & miR-192 gene expression in plasma and liver tissue samples.

Groups	miR-122		miR-192	
	Plasma	Liver Tissue	Plasma	Liver Tissue
Group C	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00	0.00 \pm 0.00
Group H	0.45 \pm 0.08 ^a	0.18 \pm 0.08 ^a	0.88 \pm 0.10 ^{aa}	0.23 \pm 0.08 ^a
Group D	1.23 \pm 0.35 ^{aa, b}	0.91 \pm 0.30 ^{a, b}	1.85 \pm 0.52 ^{aa, b}	1.27 \pm 0.45 ^{a, b}
Group HD	2.89 \pm 0.41 ^{aa, bb, c}	2.72 \pm 0.74 ^{aa, bb, c}	3.72 \pm 0.82 ^{aa, bb, c}	3.11 \pm 0.79 ^{aa, bb, c}
Group HDL	4.98 \pm 1.11 ^{aa, bb, cc, d}	4.36 \pm 0.89 ^{aa, bb, cc, d}	6.09 \pm 0.97 ^{aa, bb, cc, d}	6.35 \pm 1.30 ^{aa, bb, cc, d}

Data are presented as Mean \pm SE (standard error); Group C: Control group; Group H: Hypoxia group; Group D; Desflurane group; Group HD: Hypoxia + Desflurane group; Group HDL: Hypoxia + Desflurane + Laparotomy group. ^a p value is significantly different comparing with C group. ^b p value is significantly different comparing with H group. ^c p value is significantly different comparing with D group. ^d p value is significantly different comparing with HD group. One initial: p value $<$ 0.05, two initials: p value $<$ 0.01.

Table 2. Mean values of plasma AST, ALT, GGT.

Groups	AST (U/L)	ALT (U/L)	GGT (U/L)
Group C	128.83 ± 13.68	104.50 ± 10.13	25.33 ± 1.02
Group H	150.83 ± 16.80	120.83 ± 13.27	32.83 ± 4.43
Group D	138.50 ± 10.21	103.67 ± 11.80	29.50 ± 3.59
Group HD	359.17 ± 15.78 ^{aa,bb,cc}	231.50 ± 19.79 ^{a,bb,cc}	46.17 ± 9.76 ^a
Group HDL	488.67 ± 59.73 ^{aa,bb,cc,d}	377.00 ± 57.38 ^{aa,bb,cc,d}	70.67 ± 6.77 ^{aa,bb,cc,d}

Data are presented as Mean ± SE (standard error); Group C: Control group; Group H: Hypoxia group; Group D; Desflurane group; Group HD: Hypoxia + Desflurane group; Group HDL: Hypoxia + Desflurane + Laparotomy group. AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; GGT: Gamma-glutamyl transferase. ^a *p* value is significantly different comparing with C group. ^b *p* value is significantly different comparing with H group. ^c *p* value is significantly different comparing with D group. ^d *p* value is significantly different comparing with HD group. One initial: *p* value <0.05, two initials: *p* value <0.01.

Table 3. Frequency and Percentage of inflammatory grades, apoptosis, and necrosis.

Groups	Inflammatory Grade 1	Inflammatory Grade 2	Apoptosis	Necrosis
Group C	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)
Group H	3(50.0%) ^{aa}	0(0.0%)	1(16.7%) ^{aa}	0(0.0%)
Group D	2(33.3%) ^{aa, bb}	0(0.0%)	1(16.7%) ^{aa}	0(0.0%)
Group HD	4(66.7%) ^{aa, bb, cc}	0(0.0%)	2(33.3%) ^{aa, bb, cc}	0(0.0%)
Group HDL	1(16.7%)	3(50.0%) ^{aa,bb,cc,dd}	2(33.3%) ^{aa, bb, cc}	0(0.0%)

Data are presented as frequency and percent. C: Control group; H: Hypoxia group; D; Desflurane group; HD: Hypoxia + Desflurane group; HDL: Hypoxia + Desflurane + Laparotomy group. *P* value <0.01. ^{aa} *p* value is significantly different comparing with C group. ^{bb} *p* value is significantly different comparing with H group. ^{cc} *p* value is significantly different comparing with D group. ^{dd} *p* value is significantly different comparing with HD group.

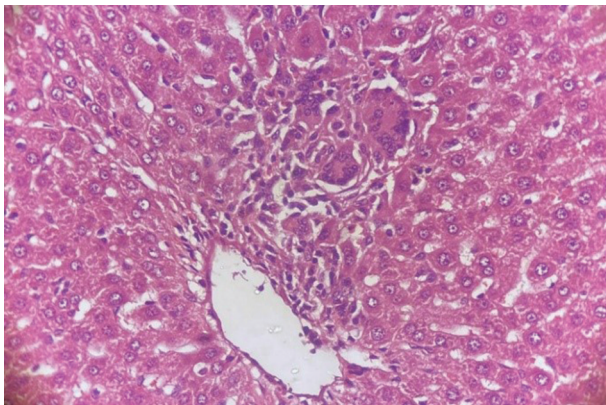


Figure 1. Slide showing centrilobular granulomatous inflammatory infiltrate showing multiple multinucleated giant cells, histocytes, and small lymphocytes, H&E (hematoxylin & eosin) x400.

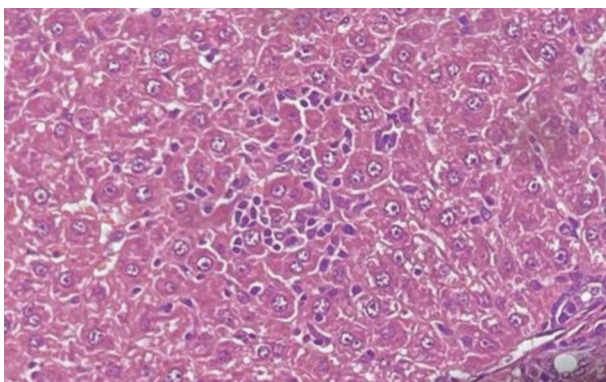


Figure 2. Slide showing apoptotic hepatocytes with surrounding lymphocytic infiltrate, H&E (hematoxylin & eosin) x400.

Table 4. The correlations between genomic biomarkers, liver functions, and histopathological findings.

	miR-122 in Plasma	miR-122 in Tissue	miR-192 in Plasma	miR-192 in Tissue
miR-122 in Tissue	0.826**			
miR-192 in Plasma	0.893**	0.881**		
miR-192 in Tissue	0.786**	0.869**	0.821**	
AST U/L	0.594**	0.629**	0.547**	0.600**
ALT U/L	0.361*	0.485**	0.429*	0.533**
GGT U/L	0.515**	0.644**	0.515**	0.677**
Inflammation	0.279	0.353*	0.359*	0.355*
Apoptosis	-0.018	-0.059	-0.046	0.038

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

to the inflammatory infiltration. Conversely, there is a non-significant negative correlation between them and apoptosis (Table 4).

4. Discussion

Drug-induced liver injury (DILI) was judged via hepatic genomic biomarkers, that is, miRNA-122, and miRNA-192 [18–21] but to our knowledge, inhalational anesthetics are not involved in such studies providing novelty to this study. The privilege of this experimental study is the capability of examining each element separately (hypoxia, desflurane, and laparotomy) with its potential hepatic affection, nevertheless it is quite difficult to be pragmatic in humans.

This experiment showed that desflurane and hypoxia, each of them induced hepatic deleterious effects that increased by their combination and further aggravated by adding the surgical influence. These changes were detected by evaluating genomic biomarkers levels (plasma and liver miR-122 and -192) and inflammatory and apoptotic changes of liver histopathology. However, the liver transferase enzymes (ALT, AST, and GGT) were comparable among desflurane, hypoxic, and control groups. These genomic biomarkers in plasma correlated with those in liver tissue and with liver enzymes as well as the inflammatory grades of histopathological findings having the advantages of being specific, and noninvasive.

The current study revealed that the plasma and liver tissue level of miR-122 and miR-192 gene expression significantly elevated in an ascending manner in groups H, D, HD, and HDL. Liver transferase levels were significantly elevated in HD and HDL groups being higher in the latter group in comparison to the other groups. This could be attributed to that the hypoxic changes can provoke immediate elevations of liver enzymes rather than the delayed immune allergic reactions of desflurane, which need longer to be elicited. These findings demonstrated the superiority of genomic biomarkers in early detection of liver affection [22].

The results of the present research go in accordance with those of the former studies [15,23–25] which stated that the biomarker miR-122 is more sensitive than ALT responsive to the drug dose and exposure period of DILI and for early diagnosis of hepatic affection during follow-up period. Furthermore, this finding was also observed on administration of chemical, special diets [26], herb-induced liver injury [27], and following administration of compounds associated with potential liver damage as phenobarbital, allyl alcohol, alpha-naphthyl isothiocyanate, and acetaminophen [28]. Inherently, miRNAs are steadier, easier to extract and analyze in all serum samples as well as persist for longer time in organic samples than other proteins. Recently, miRNAs have evolved to be confident goals for therapeutic advancement in cancer patients [29].

Werner et al. [17] compared sham laparotomy and partial hepatectomy in rats versus isoflurane anesthesia as a positive control group but without negative control group (untested). They examined about 323 miRNAs and found that in liver tissue, deregulation occurred in 15% of them after partial hepatectomy and 14% following sham laparotomy. In plasma, miRNA increase was detected for miR-133a and -133b following partial hepatectomy and sham laparotomy that could be referred to surgical stress or trauma rather than direct effect of hepatectomy.

Zhang et al. [30] performed partial hepatectomy as a traumatic model of hepatic injury and assessed the serum miRNAs in rats, but their study did not include

sham operation. They demonstrated a rise in twenty-one miRNAs. They found a 70-fold upregulation of miR-9 expression indicating their sensitivity over liver enzymes and C-reactive protein for traumatic liver injury.

Another study conducted by Soubhia et al. [16] who compared the effects of sevoflurane and halothane on hepatic injury model in rats distributed in 5 groups; the first control group was not exposed to any interference; however, rats in the remaining four groups were given phenobarbital to induce hepatic injury; three groups of them were exposed to hypoxic gas mixture to resemble ischemic effects; then the last two groups were exposed to either 1% halothane or 2% sevoflurane. They did not study the effect of surgery nor the effect of anesthetic agent alone on hepatic integrity. They displayed that the mean values of transferase enzymes in the control, phenobarbital, and hypoxia groups were similar. On the other hand, their levels were higher in the halothane and sevoflurane groups but cannot differentiate the hepatotoxic effects between both anesthetics. These differences may require more sensitive markers to detect early liver affection as approved by their optical microscopical examination, which revealed that sevoflurane had significant lower incidence of steatosis, inflammatory infiltrate, and necrosis when compared to halothane.

Even the statement that sevoflurane may be efficiently less hepatic injurious than other anesthetics such as halothane, enflurane, or desflurane [31,32], should be approved by hepatic function assessment via those genomic biomarkers. Furthermore, addition of nitrous oxide to desflurane anesthesia did not alter the gene, the inflammatory profile, or the neuroendocrine response in surgical patients [33].

In the present study, histopathological findings of the liver tissue revealed inflammatory infiltration in 33.3% of group D, 50% of group H, 66.6% of group HD and HDL. Though, all the groups showed grade one inflammatory changes, but group HDL displayed grade one in 16.7% and grade two in 50%. Concerning apoptosis, 16.7% were shown in groups H and D each and 33.3% in groups HD and HDL each, while none of the groups exhibited necrosis.

Zhang Y et al. [34] results agreed with ours as they reported that the increase in plasma miR-122 level was greater and earlier than the changes in ALT and before hepatic histopathologic changes in mice induced liver injury by D-galactosamine and alcohol. Meanwhile, Jarsiah et al. [23] reported some histopathological changes in the rats' liver injected with acetaminophen as mild edema, portal hyperemia, mild inflammatory cells infiltration, and mild centrilobular spotty necrosis of liver cells.

Limitations of this study are the small sample size and additional group may be needed to detect the effect of phenobarbital in a separate group.

This experimental study concluded that desflurane and hypoxia, each of them has deleterious effect on hepatic integrity, which increased by their combination and aggravated by surgical influence. These effects were established by measuring miR-122 and miR-192, and histopathological examination that were correlated with each other. These results could not be detected by liver transferase enzymes. Further study with calculated sample size is required. Moreover, assessment of hepatotoxic effects of other various anesthetic agents and different surgical approaches in experimental study using hepatic genomic biomarkers are recommended. Furthermore, these biomarkers are recommended to be applied clinically for perioperative detection of early hepatic affection in susceptible cases.

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Ethics committee approval

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