Heart rate variability and its impact on pulmonary dysfunction in rats subjected to hemorrhagic shock

Fateme Khodadadi

Shiraz University

Aminollah Bahaoddini

Shiraz University

Alireza Tavassoli

Fasa University of Medical Science

Farzaneh Ketabchi (✉ ketabchif@sums.ac.ir)

Shiraz University of Medical Sciences <https://orcid.org/0000-0002-1157-3299>

Research article

Keywords: HRV, Lung injury, Shock, Vagus nerve

Posted Date: November 2nd, 2019

DOI: <https://doi.org/10.21203/rs.2.16424/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Introduction: The activity of autonomic nervous system and its association with organ damage have not been entirely elucidated in hemorrhagic shock. The aim of this study was to investigate heart rate variability (HRV) and pulmonary gas exchange in hemorrhagic shock during unilateral subdiaphragmatic vagotomy.

Methods: Male Sprague Dawley rats were randomly assigned into groups of sham, vagotomized (Vag), HS and Vag+HS. HS was induced in conscious animals by blood withdrawal until reaching to mean arterial blood pressure (MAP) of 40 ± 5 mm Hg. Then, it was allowed to MAP returning toward the basal values. MAP and heart rate (HR) were recorded throughout the experiments, HRV components of low (LF, sympathetic index), high (LH, parasympathetic index), and very low (VLF, injury index) frequencies and the LF/HF ratio (sympathetic index) calculated, and the lung histological and blood gas parameters assessed.

Results: In the initial phases of HS, the increase in HR with no change in MAP were observed in both HS and Vag+HS groups, while LF increased only in the HS group. In the second phase, HR and MAP decreased sharply in the HS group, whereas, MAP decreased only in the Vag+HS group. Meanwhile, LF and HF increased relative to their baselines in the HS and Vag+HS groups, even though the values were much pronounced in the HS group. In the third phase, HR, MAP, LF, HF, and the LF/HF ratio were returned back to their baselines in both HS and Vag+HS groups. However, in the Vag+HS group, the VLF was lower and HR was higher than those in the other groups. Furthermore, blood gas parameters and lung histology indicated the impairment of gas exchange in the Vag+HS group.

Conclusions : The sympathetic activity is predominant in the first phase, whereas the parasympathetic activity is dominant in the second and third phases of hemorrhagic shock. Furthermore, hemorrhagic shock with vagotomy may be linked to lung injury and decreased VLF.

Introduction

Hemorrhagic shock (HS) is one of the leading causes of death in the world [1]. The inability to supply the oxygen demand of the body organs in HS may lead to a severe cell damage, metabolic disorders, or even cell death in untreated patients [2]. HS is categorized into four classes. In classes I and II, despite the blood loss, systolic blood pressure, pulse pressure, and plasma levels of bicarbonate and lactic acid remain in the normal range or may change slightly. The constant blood pressure can be linked to the increase in heart rate (HR) or vascular resistance because of the neural activity and humoral factors [3]. A substantial decrease in cardiac output and blood pressure occurs by 30–40% of blood loss in reversible class III. However, if hemorrhage persists without any therapeutic intervention, the compensatory mechanisms may be failed which results in deadly irreversible shock in class IV [4].

The hemodynamic responses to blood loss in classes I and II of hemorrhagic shock in conscious animals consist of three phases [5]. In the initial compensation phase, the arterial blood pressure is maintained

despite a decrease in cardiac output [6]. In the second phase namely decompensatory phase, both arterial blood pressure and HR drop sharply in parallel with losing 15 to 20% of total blood volume [7]. In the third phase or recompensatory phase, the arterial blood pressure, and HR recover gradually when the hemorrhage is terminated [8]. However, mechanisms of different phases of compensation have not been fully elucidated thus far.

The compensatory response to hemorrhagic shock depends on the integrity of afferent fibers of the vagus nerve originate from the arterial baroreceptors [5, 9, 10]. Furthermore, the subdiaphragmatic vagus nerves may be involved in regulating the autonomic nervous system's negative feedback [11]. It may also play a role in immune responses through anti-inflammatory cholinergic pathways [12]. However, no study has clarified the role of subdiaphragmatic vagus nerves in class II hemorrhagic shock.

Inappropriate treatment of hemorrhagic shock may lead to body tissue and multi-organ failures. The lung is very vulnerable to the damage because it receives the total cardiac output per minute. Therefore, it may be affected by many substances, which are released from the injured tissues passing through the circulation to the lung. In a few studies, the increase in pulmonary capillary permeability and the infiltration of immune cells into the pulmonary interstitial space have been shown in hemorrhagic shock [13]. However, the role of subdiaphragmatic vagus nerve in lung injury induced by hemorrhagic shock remains unclear.

The time intervals between two consecutive heartbeats are frequently influenced by the activity of autonomic nervous system, physical activity, metabolism and homeostatic challenges [14]. These fluctuations are defined by analyzing heart rate variability (HRV) [15]. The analyses of HRV may provide useful information about the autonomic nervous system activity in three phases of compensation in HS. To date, a few studies have indicated HRV can reflect the autonomic activity variations in the early and compensatory phases of shock [16]. A study by Porter et al. identified that HRV depends on the rate of bleeding [17]. HF band is an index for parasympathetic activity and respiratory cycle-related heart rate [18]. LF band is an index of the activities of sympathetic system and baroreceptors [15, 19]. The LF/HF ratio is used to assess the sympathetic balance and VLF is related to the metabolic regulatory mechanisms and injury [20, 21].

Based on the results of the noted researches, the present study aimed at investigating the alterations of hemodynamic and HRV at three phases of class II hemorrhagic shocks in conscious rats. The relation between the HRV, lung tissue damage and blood gas parameters in the three phases of hemorrhagic shock were evaluated. Furthermore, the role of the subdiaphragmatic vagus nerve was assessed at the above conditions. This study was designed in conscious rats to exclude the effect of anesthetic drugs on the respiratory system-related heart rate variability.

Methods

Study design

This study was conducted based on the Medical Ethics Committee Regulations in accordance with the declaration of Helsinki on 24 male Sprague-Dawley rats weighing 250–300g. Animals were housed in standard cages under controlled laboratory temperature, humidity, and 12:12 hours of light/dark cycles. They had free access to water and standard food, and were fasted a few hours before starting the experiments, and randomly assigned into four groups of Sham (n = 5), vagotomized (Vag, = 5), hemorrhagic shock without (HS, n = 7) and with vagotomy (HS+Vag, n = 7). Animals were anesthetized by intraperitoneal injection of 50 mg/kg sodium pentobarbital (Sigma). Additional doses used if needed. The femoral vein was then cannulated by a 120-PE catheter. It was passed through a hole in the rear skin of the tail, and fixed by two sutures. Then, a 50 PE catheter was inserted into the tail artery, and fixed firmly. Thereafter, the area of surgery was rinsed by 1% lidocaine (Sigma) to minimize the postoperative pain. Then, the conscious animals were transferred to a dark metabolic cage with a constant temperature. In order to manage the stress and anxiety of animals, the experiments were performed under a calm with minimum noise conditions. Animals' tails were fixed outside the cage. Therefore, they could relatively move in a cage without damaging the catheter, and disturbing the recording setup. The arterial catheter was connected through a pressure transducer (MLT844) to a data acquisition system (Powerlab, PL26T04, ADInstruments, Australia). The mean arterial blood pressure (MAP) was recorded throughout the experiments and HR calculated accordingly. The femoral vein was used for blood sampling, and blood withdrawal during induction of hemorrhagic shock.

Subdiaphragmatic vagotomy

After anesthesia, an incision was made in the upper abdominal skin. The fascia and muscles were dissected, and the left subdiaphragmatic vagus nerve, separated from the surrounding tissues, and cut apart. Then, muscles and skin were sutured, and the surgical areas were rinsed with 1% lidocaine. In the Sham group, animals underwent the arterial and venous cannulation and abdominal laparotomy, whereas, the vagus nerve remained intact.

Study protocol: Induction of hemorrhagic shock

After 70 minutes of the steady-state period, the arterial and venous blood samples were taken for analyzing the blood gas parameters. Then, groups of HS and Vag+HS were subjected to hemorrhagic shock, according to the previous studies and our pilot experiments [17]. Briefly, the blood withdrawal with a constant flow rate (0.5 ml/min/rat) was taken from the femoral vein until the mean blood pressure reached to 40 ± 5 mm Hg. After that, the blood withdrawal was ceased so that it was allowed MAP returned to the pre-hemorrhage level. The amount of blood volume (BV) was estimated using the Lee and Blafox equation:

$$\text{EBV ml} = 0.06 \times \text{body weight g} + 0.77 \text{ [22]}.$$

Two hours later, the arterial and venous blood samples were taken for blood gas analysis. MAP and HR were recorded continuously throughout the experiments. Finally, animals were killed by high doses of pentobarbital and the lungs were prepared for histological analyses. All mentioned procedures were performed in the groups of Sham and Vag in the same time course as in the HS groups, except for the induction of hemorrhagic shock (Figure 1).

HRV Analysis

Kubios HRV Premium/Animal software (ver. 3.2) was used for HRV analysis. The interpolated tachogram of 10 Hz [23], the Welch's period diagram window width for 512 pieces and the hanning window with 50% overlap were selected. Based on previous studies, frequency-domain components of very low (VLF: 0 to 0.2 Hz), low (LF: 0.20 to 0.75 Hz), high-frequency (HF: 0.75 to 3.0 Hz) [24, 25], and LF/HF ratio were considered in this study. We used HRV analysis in three phases of class II hemorrhagic shock. The duration of calculations in the first and third phases was within 5 minutes, and in the second phase was for one minute. At each time interval, the pulse interval was calculated offline from the intervals of the peak systolic blood pressure. Interval pulse data were exported from the Kubios software to the Excel. Finally, the HRV components of LF, HF, and VLF data were expressed as logarithm values (log).

Arterial and venous blood gas parameters

The 200 µl of blood samples were taken during the baseline period, and at the end of the experiments for the blood gas analyses using an easy blood gas device (Medica, USA). The arterial and venous oxygen contents were calculated based on the following equation.

$CaO_2 = \text{oxygen bound to Hb} + \text{oxygen in dissolved state} = (1.34 \times \text{Hb} \times SaO_2) + (PaO_2 \times 0.003)$; $CvO_2 = \text{oxygen bound to Hb} + \text{oxygen in dissolved state} = (1.34 \times \text{Hb} \times SvO_2) + (PvO_2 \times 0.003)$.

Histological evaluations

At the end of the experiments, the chest was opened and the lung removed and fixed in 4% formalin. Then, the samples were dehydrated by different concentrations of ethanol and xylol and embedded in paraffin. Tissue sections were prepared by a microtome (pfm medical, UK) and stained with hematoxylin and eosin [13]. All slides of histology were evaluated in a blinded manner by a pathologist. The pathological components of the focal thickening of the alveolar membranes, vascular congestion, perivascular neutrophil infiltration, and alveolar hemorrhage were evaluated. Each index was graded with a score of 0 to 3 based on the absence (0), mild (1), moderate (2) or severe (3) [13].

Statistical Analysis

Data are given as mean $\pm$ SE. Analysis of variance (ANOVA) with Tukey's post hoc test was used for multiple comparisons. Also, repeated-measure ANOVA was used for comparison of data during the different time course of experiments. We used the paired T-Test for comparing the data of blood gas variables at the onset and offset of the experiments, and other variables if needed. Furthermore, we compared data of histology using the non-parametric Mann-Whitney test. All analysis was performed using the software of SPSS 18. Significance was assumed when $P < 0.05$ and the confidence limits used were the 95% intervals.

Results

Animals in the HS and Vag+HS groups underwent hemorrhage with the volume of $26.07 \pm 3.07\%$ and $23.37 \pm 1.68\%$ of EBV, respectively. Also, the duration of blood withdrawal were 8.92 ± 0.94 and 8.42 ± 0.52 minutes in the HS and Vag+HS groups, respectively. No significant difference was detected in the amount or duration of blood withdrawal at above-mentioned groups.

Figure 1 demonstrates the real traces of blood pressure (BP) and HR in the experimental groups. The time courses of the experiments in all groups were identical. Three phases of the initial (In-Com), decompensatory (De-Com) and recompensatory (Re-Com) have been highlighted. As shown in Figure 1 and Figure 2, the arterial blood pressure and heart rate in the Sham and Vag groups did not alter significantly throughout the experiments.

In the HS group, MAP was maintained in the normal range during the In-Com phase. It then decreased, reaching to a minimum value of 41.5 ± 1.5 mm Hg in the De-Com phase. After cessation of blood withdrawal, MAP returned slowly to its baseline level in the Re-Com phase. Unlike the MAP, HR was high in the In-Com phase of the HS group compared with the baseline ($p \leq 0.05$). However, in the De-Com phase, MAP dropped sharply along with a sudden drop in HR, compared with their baselines ($p \leq 0.01$). Also, MAP in this phase was lower than the ones in the Sham and Vag groups ($p \leq 0.01$). There was no significant alteration between the values of MAP and HR during the Re-Com phase in the HS compared with their baseline values (Figure 2).

No variation was detected in the baseline values of MAP and HR in the Vag+HS group. Also, the pattern of those variables in the In-Com phase was similar to those in the HS group. That is, MAP was maintained and HR was higher than those in their baselines ($p \leq 0.01$). In the De-Com phase, alterations of MAP in the Vag+HS group were similar to that in the HS group. However, unlike the HS group, the HR in the Vag+HS group did not show a significant difference with respect to its baseline, or those ones in the Sham and Vag groups. MAP in the Re-Com phase of the Vag+HS group was similar to that in the HS group. Nevertheless, HR in the Vag+HS group increased gradually being higher than those in the Sham ($p \leq 0.01$) and Vag ($p \leq 0.05$) groups at 30 minutes of the Re-Com phase until the end of the experiments. There was no significant difference in MAP after minute 50, to the end of experiments between the HS and Vag+HS groups. However, HR showed higher values in the Vag+HS group compared with those in the HS group during the last 30 minutes of the Re-Com phase ($p < 0.05$) (Figure 2).

The HRV components did not show significant difference in all groups at baseline. In the In-Com phase, LF and HF increased significantly ($p \leq 0.05$). Furthermore, there was a slight increase of VLF in the In-Com phase of the HS group. The increases of LF, HF and VLF were much pronounced in the De-Com phase as compared with their baselines, and that in the Sham group ($p \leq 0.01$), and returned slowly to their baselines in the Re-Com phase. The patterns of LF and HF during the De-Com and Re-Com phases in both HS and Vag+HS groups were similar, though the values in the Vag+HS group were lower than those in the HS group. Also, the increase in VLF in the De-Com phase of the Vag+HS group was significantly lower than that in the HS group ($p \leq 0.01$). The VLF was also lower in minute 20 and minutes 60–110 in the Vag+HS group compared with those in the HS group ($p \leq 0.05$). The LF/HF ratio showed a significant reduction in the De-Com phase of the Vag+HS group as compared with the other groups ($p \leq 0.05$), and was higher than the baseline in minutes 100–110 ($p \leq 0.05$) (Figure 3).

Table 1, and Table 2 indicate the arterial and venous blood gas parameters taken at the end of the steady-state period, and at the end of the experiments. Because the atmospheric pressure is about 630 mm Hg at the site where the experiments were conducted, PaO_2 of 65 ± 5 mm Hg is considered as normal. There was no difference in all values at the baseline between groups. At the end of the experiments, the arterial oxygen pressure (PaO_2) in the Vag+HS group was lower than that in the Sham group ($p \leq 0.05$). Also, the venous oxygen pressure (PvO_2) in the Vag+HS group was lower than those in the Sham and Vag ($p \leq 0.05$). There was no significant difference among other variables at the onset to the end of the experiments in other groups.

At the end of the experiments, the Hb concentrations in both HS groups were lower than those in the Vag and Sham groups. However, it was only significant in the Vag+HS group compared with the Vag group. The $\text{PaO}_2/\text{FIO}_2$ ratio in the Vag+HS group was lower than that in the Sham group (normal value of $\text{PaO}_2/\text{FIO}_2$ ratio is about 300 ± 10 mm Hg) ($p \leq 0.05$). Also, the decrease in PaO_2 were along with the decrease in arterial oxygen content (CaO_2) ($p \leq 0.05$, compared with the Sham and Vag groups). In addition, the venous oxygen content (CvO_2) in the HS group was lower than those in the Sham and Vag group ($p \leq 0.05$) and CvO_2 in the Vag+HS group was lower than those in the Sham and Vag group ($P \leq 0.01$) (Table 3).

Vascular congestion in the Vag+HS group was higher than those in the Sham, Vag and HS groups ($p \leq 0.05$). There was no alteration between histological parameters between the Sham, Vag, and Vag+HS groups (Figure 4).

Discussion

A few studies have reported the spectral analysis of HRV, and the role of subdiaphragmatic vagus nerve in class II hemorrhagic shock in conscious animals. Besides, the impact of HRV and acute lung injury has not been illustrated at the mentioned conditions [26]. Therefore, in the present study, hemodynamic parameters and HRV were evaluated during three phases of class II hemorrhagic shock. Furthermore, the association between the level of the autonomic nervous system activity and lung injury was evaluated.

The contribution of autonomic nervous system differs in three phases of class II hemorrhagic shock. The sympathetic activity in the In-Com phase shifts to the parasympathetic activity in the De-Com phase. Also, the parasympathetic activity is much pronounced in the Re-Com phase of hemorrhagic shock. Although subdiaphragmatic vagotomy does not change the baseline hemodynamic parameters, it increases the heart rate during the hemorrhagic shock, particularly in the Re-Com phase, and leads to lung injury probably linked to interfering with anti-inflammatory effect of the cholinergic pathway.

No alteration was identified in the values of MAP and HR at baseline. Therefore, all groups enter the study with identical conditions. In addition, no variation in the MAP and HR between the vagotomized and non-vagotomized animals, suggest the subdiaphragmatic vagus nerve may not regulate directly the systemic hemodynamic. In fact, in order to minimize the direct effect of the vagus nerve on hemodynamic and heart rate variability components, we did subdiaphragmatic vagotomy instead of cervical vagotomy. Likewise, no alteration was detected in MAP and HR during the time course of experiments in the Sham and Vag groups. Therefore, the unilateral subdiaphragmatic vagotomy per se could not affect the hemodynamic and cardiac function.

The constant arterial blood pressure in the In-Com phase of blood withdrawal in the HS group can be due to the increase in HR, which is also supported by the results of previous studies [5]. Other studies have indicated that the systemic vascular resistance, but not HR, increases markedly in conscious rats subjected to hemorrhagic shock [8]. However, in our study, we did not measure vascular resistance because of limitations in the experimental preparations. Therefore, it can be suggested that at least a part of a constant blood pressure is linked to the increase in HR. There was not a significant variation in the parasympathetic component of HF, though it tended to increase. However, a significant increase in LF may be due to the increase in sympathetic activity [19]. On the other hand, the LF/HF ratio showed no change, which can be related to an insignificant increase in HF. Together, it is expected that the parasympathetic activity decreases and the sympathetic activity increases in this phase. In line with our results, Porter et al. showed that the increase of HR in the compensatory phase of hemorrhage, is mainly mediated by the increase in sympathetic activity, but not the parasympathetic drive [17]. By contrast, Troy et al have reported that the constant heart rate in the initial phase of hemorrhagic shock is related to the decrease in parasympathetic drive, with no change in the sympathetic activity [5]. The difference in the experimental conditions may be influential in this dissimilarity results.

In our study, the unilateral subdiaphragmatic vagotomy did not alter the MAP in the

In-Com phase in the Vag+HS group, whereas it increased HR. There was no difference between LF and HF in the Vag+HS group compared with those in the Vag and Sham groups. Therefore, the increase in HR may be linked to the decrease in parasympathetic activity, increase in sympathetic activity, or both.

The blood withdrawal resulted in a sudden drop in MAP and HR in the De-Com phase of the HS group similar to some studies [5], whereas, others have reported the opposite results [27]. HF increased in the De-Com phase of the HS group in agreement with the report of Porter et al [17]. Besides, an increase in LF was observed in the De-Com phase of HS in our study, causing the LF/HF ratio to remain unchanged. The

increase in LF may be related to the increase in sympathetic activity. This conclusion is in contrast with the reports of Potas & Dampney that the sympathetic system is inhibited in hemorrhagic shock linked to the reduction of venous return and cardiac filling, and leads to the reduction of heart rate [28]. However, the question remains why the increase in LF was not associated with the increase of HR in our study. There are some evidences, indicating that the LF band cannot be considered entirely as a sympathetic index. In one study, it has been reported that when parasympathetic activity is high, the LF band cannot be used as a reliable index for the sympathetic activity [29]. Furthermore, angiotensin II increases a few minutes after induction of hemorrhagic shock. Angiotensin II increases the LF band, whereas, administration of angiotensin II receptor antagonist has an opposite effect [30, 31]. Therefore, it can be argued that at least parts of increased LF band in the De-Com phase of both hemorrhagic shock groups are linked to the increases in plasma angiotensin II. To sum up, since the parasympathetic index of HF increases and HR decreases in the De-Com phase of HS, the dominant effect of the parasympathetic activity is suggested in this phase.

Although, the reduction in MAP in the De-Com phase of both HS and Vag+HS groups were similar, HR did not decrease in the Vag+HS group. However, the amount of blood withdrawn in this group was identical to that in the HS group. Therefore, it can be suggested that the main cause of pressure drop in this phase may be the decrease in vascular resistance, but not reduced HR. We did not find a similar study indicating the effect of subdiaphragmatic vagotomy on HRV components during hemorrhagic shock. However, it has been shown that any drug, surgery, or physiological interventions which disrupts the parasympathetic activity could reduce HRV [32]. Also, the subdiaphragmatic vagal nerve contains both afferent and efferent fibers that may play a role in regulating the negative feedback in sympathetic activity and adrenal medullary secretion. Thus, the interference in vagal subdiaphragmatic pathways may increase the plasma epinephrine following the disinhibition of the adrenal gland [11]. Consequently, it may prevent of bradycardia during the De-Com phase of hemorrhage in the Vag+HS group. Furthermore, angiotensin II released in this phase may decrease the R-R interval and increase HR [31]. As a result, increased HR in the De-Com phase of the Vag+HS group may be linked to the effects of angiotensin II and epinephrine. On the other hand, HF and LF were high in the De-Com phase of the Vag+HS group, even though these parameters were lower than those in the HS group. Also, the LF/HF ratio was low in the Vag+HS group. Taken together, the decrease in MAP, with no change in HR, concomitant with the results of HRV analyses suggest the less sympathetic activity compared with the parasympathetic activity in the De-Com phase of class II hemorrhagic shock.

In the third phase, which lasted for 110 minutes, the HR and MAP gradually returned back to the baseline in the HS group, and did not indicate a significant difference with the ones in the Sham group. All components of HRV were gradually recovered. Although at the end of the record, LF appeared to be higher than those in the Sham group and their baselines, these differences were not significant. A few studies have addressed the hemodynamic alterations and HRV in the Re-Com phase of hemorrhagic shock. In the study of Porter et al. the recovery was assessed within 30 minutes after hemorrhage [17], which was not enough time to return MAP and HR to their baselines. Our study was performed in a long time, so that the hemodynamic parameters returned to their baselines and being stable up to the end of the experiments.

However, HR increased after minute 10 of Re-Com phase in the Vag+HS group, being significant from 30 minutes to the end of the experiments. There was no significant variation between HF during the time course of Re-Com phase in the Vag+HS group. As a result, the cause of increased HR cannot be directly linked to the decrease in the parasympathetic drive. It may be related to the effect of subdiaphragmatic vagotomy on the medullary adrenal gland and the release of norepinephrine [11]. In addition, the LF/HF ratio at the end of experiments in the Vag+HS group was higher than that in the HS group, which may suggest a gradual increase in sympathetic drive in this time.

Our results showed a significant increase in VLF in the De-Com phase and at the onset of the Re-Com phase, and it returned to the baseline level thereafter in the HS group. Although, the VLF increased in the De-Com phase of Vag+HS group, it was significantly lower than that in the HS group. However, VLF returned to the baseline level in the Vag+HS group faster than the HS group. Our data suggest that the relationship between the HR and the level of VLF could be inversely proportional. The decrease in VLF leads to the increase in HR. The increase in VLF band may be also due to the increase of the renin angiotensin system activity, temperature regulatory mechanisms [33] or endocrine and hypothalamic systems involved in metabolic rate [21]. Numerous studies have suggested that among the HRV components, the VLF band is a powerful indicator in predicting different diseases and injuries [34]. In our study, VLF in the Re-Com phase of the Vag+HS group was lower than that in the Sham group. Therefore, it can be suggested that there is an inverse relationship between the level of VLF and organ dysfunctions including the lung in hemorrhagic shock.

There was no significant alteration in the blood gas parameters at the end of the experiments in the Sham, Vag and HS groups. However, the subdiaphragmatic vagotomy resulted in the decreases in PaO_2 and PvO_2 in the Vag+HS group. Also, the $\text{PaO}_2/\text{FIO}_2$ ratio in the Vag+HS group was lower than that in the Sham group. Since the histological assessments indicated vascular congestion in the Vag+HS group, it can be proposed that the reduction of PaO_2 and $\text{PaO}_2/\text{FIO}_2$ ratio in the Vag+HS group is linked to shunt or ventilation/perfusion mismatching in the lung [35].

The Hb concentration in both hemorrhagic groups of HS and Vag+HS groups was lower than those in the Sham and Vag groups, though it was only significant in the Vag+HS group. Volume resuscitation has not been performed in this study. Therefore, the low concentration of Hb can be linked to the absorption of fluid into the blood capillaries from the interstitial spaces of the body tissues and dilution of the blood as a normal compensatory mechanism [36]. Furthermore, the decrease in PaO_2 and PvO_2 along with a decrease in hemoglobin concentration resulted in the decreases in CaO_2 and CvO_2 in the Vag+HS group.

In the hemorrhagic shock, the arterial-venous oxygen content difference increases due to the increased oxygen demand of the affected tissues. Therefore, the decrease in PvO_2 in the Vag+HS group along with increased the arterial-venous oxygen content difference may be linked to the increase in tissue oxygen uptake by body organs including the heart in order to repaying the oxygen debt [37].

Conclusions

In this study, we indicated the contribution of autonomic nervous system in three phases of class II hemorrhagic shock. The sympathetic system is predominant in the first phase, whereas the parasympathetic system is dominant in the second and third phases of hemorrhagic shock. Also, the subdiaphragmatic vagotomy leads to lung injury in hemorrhagic shock. Taken together, the analyzing the HRV during different phases of hemorrhagic shock may pave the way for rapid diagnosis and treatment of the patients during critical condition in the hospital. Also, the administration of anti-cholinergic drugs may not be recommended because of worsening the lung injury.

Declarations

Abbreviations

HS: hemorrhagic shock; Vag: vagotomy; HRV: heart rate variability; VLF: very low frequency; LF: low frequency; HF, high frequency; MAP: mean arterial blood pressure; HR: heart rate; *Hb*: hemoglobin; PaO₂: arterial oxygen pressure; PvO₂: venous oxygen pressure; CaO₂: arterial oxygen content; CvO₂: venous oxygen content; PaO₂-PvO₂: arterial-venous oxygen pressure difference; CaO₂-CvO₂: arterial-venous oxygen content difference; FIO₂: fractional concentration of oxygen.

Acknowledgement

The authors wish to thank the Center of Comparative and Experimental Medicine of University for providing the rats.

Authors' contribution

F.Kho and A.T performed experiments; F.Ket and F.Kho analyzed data; F.Ket, F.Kho, A.B and A.T interpreted results of the experiments; F.Kho prepared figures; F.Kho and F.Ket drafted the manuscript; F.Ket and A.B edited and revised manuscript; F.Ket, F.Kho, A.B and A.T approved final version of the manuscript.

Funding

This work is supported by the Research Council of Shiraz University of Medical Sciences, grant No 96–01–01–14378, the Research Center for Thoracic and Vascular Surgery, and the Research Council of University of Shiraz, as a part of work for acquiring an Ph.D degree in physiology by F. Khodadadi.

Availability of data and materials

The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This study was conducted based on the Medical Ethics Committee Regulations at Shiraz University of Medical Sciences, Shiraz, Iran (Reference No IR.SUMS.MED.REC.1396.s203).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interest.

Author details

¹ Dep of Biology, College of Sciences, Shiraz University, Shiraz, Iran. ² Dep of Physiology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran. ³ Dep of Pathology, Fasa University of Medical Sciences, Fasa, Iran.

References

1. Perel P, Prieto-Merino D, Shakur H, Clayton T, Lecky F, Bouamra O, Russell R, Faulkner M, Steyerberg EW, Roberts I: Predicting early death in patients with traumatic bleeding: development and validation of prognostic model. *Bmj* 2012; 345:e5166.
2. Kuai Q, Wang C, Wang Y, Li W, Zhang G, Qiao Z, He M, Wang X, Wang Y, Jiang X: Energy metabolism regulated by HDAC inhibitor attenuates cardiac injury in hemorrhagic rat model. *Scientific reports* 2016; 6:38219.
3. Orlinsky M, Shoemaker W, Reis ED, Kerstein MD: Current controversies in shock and resuscitation. *Surgical Clinics of North America* 2001; 81(6):1217–1262.
4. Scully CG, Kramer GC, Strauss DG: Evaluation of heart rate and blood pressure variability as indicators of physiological compensation to hemorrhage before shock. *Shock* 2015; 43(5):463–469.
5. Troy BP, Hopkins DA, Keay KA: The hemodynamic response to blood loss in the conscious rat: contributions of cardiac vagal and cardiac spinal signals. *Shock* 2014; 41(4):282–291.
6. Evans RG, Ventura S, Dampney RA, Ludbrook J: John Ludbrook APPS Symposium Neural Mechanisms In The Cardiovascular Responses To Acute Central Hypovolaemia. *Clinical and Experimental Pharmacology and Physiology* 2001; 28(5-6):479–487.

7. Troy B, Heslop D, Bandler R, Keay K: Haemodynamic response to haemorrhage: distinct contributions of midbrain and forebrain structures. *Autonomic Neuroscience* 2003; 108(1–2):1–11.
8. Schadt JC, Ludbrook J: Hemodynamic and neurohumoral responses to acute hypovolemia in conscious mammals. *American Journal of Physiology-Heart and Circulatory Physiology* 1991; 260(2):H305-H318.
9. Ludbrook J, Ventura S: Roles of carotid baroreceptor and cardiac afferents in hemodynamic responses to acute central hypovolemia. *American Journal of Physiology-Heart and Circulatory Physiology* 1996; 270(5):H1538-H1548.
10. Schreihofer AM, Hoffman GE, Sved AF: The kidneys stimulate vasopressin release during hemorrhage in rats with chronic NTS lesions. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 1997; 272(5):R1540-R1551.
11. Mravec B, Ondicova K, Tillinger A, Pecenak J: Subdiaphragmatic vagotomy enhances stress-induced epinephrine release in rats. *Autonomic Neuroscience* 2015; 190:20–25.
12. Rosas-Ballina M, Ochani M, Parrish WR, Ochani K, Harris YT, Huston JM, Chavan S, Tracey KJ: Splenic nerve is required for cholinergic antiinflammatory pathway control of TNF in endotoxemia. *Proceedings of the National Academy of Sciences* 2008; 105(31):11008–11013.
13. Xanthos TT, Balkamou XA, Stroumpoulis KI, Pantazopoulos IN, Rokas GI, Agrogiannis GD, Troupis GT, Demestihia TD, Skandalakis PN: A model of hemorrhagic shock and acute lung injury in Landrace–Large White swine. *Comparative medicine* 2011; 61(2):158–162.
14. Shaffer F, Ginsberg J: An overview of heart rate variability metrics and norms. *Frontiers in public health* 2017; 5:258.
15. McCraty R, Shaffer F: Heart rate variability: new perspectives on physiological mechanisms, assessment of self-regulatory capacity, and health risk. *Global advances in health and medicine* 2015; 4(1):46–61.
16. Hinojosa-Laborde C, Rickards C, Ryan K, Convertino V: Heart rate variability during simulated hemorrhage with lower body negative pressure in high and low tolerant subjects. *Frontiers in physiology* 2011; 2:85.
17. Porter K, Ahlgren J, Stanley J, Hayward LF: Modulation of heart rate variability during severe hemorrhage at different rates in conscious rats. *Autonomic Neuroscience* 2009; 150(1–2):53–61.
18. Laborde S, Mosley E, Thayer JF: Heart rate variability and cardiac vagal tone in psychophysiological research—recommendations for experiment planning, data analysis, and data reporting. *Frontiers in psychology* 2017; 8:213.

19. Lehrer PM: Biofeedback training to increase heart rate variability. *Principles and practice of stress management* 2007; 3:227–248.
20. Habek M, Crnošija L, Lovrić M, Junaković A, Skorić MK, Adamec I: Sympathetic cardiovascular and sudomotor functions are frequently affected in early multiple sclerosis. *Clinical autonomic research* 2016; 26(6):385–393.
21. Grishin O, Grishin V, Uryumtsev DY, Smirnov S, Jilina I: Metabolic rate variability impact on very low-frequency of heart rate variability. *World Applied Sciences Journal* 2012; 19(8):1133–1139.
22. Lee H, Blafox M: Blood volume in the rat. *Journal of Nuclear Medicine* 1985; 26(1):72–76.
23. Tarvainen MP, Ranta-Aho PO, Karjalainen PA: An advanced detrending method with application to HRV analysis. *IEEE Transactions on Biomedical Engineering* 2002; 49(2):172–175.
24. Cerutti C, Barres C, Paultre C: Baroreflex modulation of blood pressure and heart rate variabilities in rats: assessment by spectral analysis. *American Journal of Physiology-Heart and Circulatory Physiology* 1994; 266(5):H1993-H2000.
25. Cerutti C, Gustin M, Paultre C, Lo M, Julien C, Vincent M, Sassard J: Autonomic nervous system and cardiovascular variability in rats: a spectral analysis approach. *American Journal of Physiology-Heart and Circulatory Physiology* 1991; 261(4):H1292-H1299.
26. Salomão Jr E, Otsuki DA, Correa AL, Fantoni DT, dos Santos F, Irigoyen MC, Auler Jr JOC: Heart rate variability analysis in an experimental model of hemorrhagic shock and resuscitation in pigs. *PloS one* 2015; 10(8):e0134387.
27. Holobotovskyy VV, Arnolda LF, McKittrick DJ: Effect of anaesthetic and rat strain on heart rate responses to simulated haemorrhage. *Acta physiologica scandinavica* 2004; 180(1):29–38.
28. Potas JR, Dampney RA: Evidence that venoconstriction reverses the phase II sympathoinhibitory and bradycardic response to haemorrhage. *Autonomic neuroscience: basic & clinical* 2004; 111(1):1–6.
29. Saul JP, Rea RF, Eckberg DL, Berger RD, Cohen RJ: Heart rate and muscle sympathetic nerve variability during reflex changes of autonomic activity. *The American journal of physiology* 1990; 258(3 Pt 2):H713–721.
30. Pancera P, Presciuttini B, Sansone S, Montagna L, Paluani F, Covi G, Lechi A: Effect of losartan on heart rate and blood pressure variability during tilt test and trinitroglycerine vasodilation. *Journal of hypertension* 1999; 17(4):513–521.
31. Vaile JC, Fletcher J, Littler WA, Coote JH, Townend JN: Angiotensin II modulates cardiovascular autonomic control in the absence of baroreflex loading. *Heart (British Cardiac Society)* 1998; 80(2):127–133.

32. Billman GE: The effect of heart rate on the heart rate variability response to autonomic interventions. *Frontiers in physiology* 2013; 4:222.
33. Claydon VE, Krassioukov AV: Clinical correlates of frequency analyses of cardiovascular control after spinal cord injury. *American Journal of Physiology-Heart and Circulatory Physiology* 2008; 294(2):H668-H678.
34. Ryan ML, Ogilvie MP, Pereira BM, Gomez-Rodriguez JC, Manning RJ, Vargas PA, Duncan RC, Proctor KG: Heart rate variability is an independent predictor of morbidity and mortality in hemodynamically stable trauma patients. *Journal of Trauma and Acute Care Surgery* 2011; 70(6):1371–1380.
35. Nielsen T, Hvas C, Dobson G, Tønnesen E, Granfeldt A: Pulmonary function after hemorrhagic shock and resuscitation in a porcine model. *Acta Anaesthesiologica Scandinavica* 2014; 58(8):1015–1024.
36. Pawelczyk NS, del Pilar Huby M, Salsbury JR, Baer LA, Wang Y-WW, Matijevic N, Holcomb JB, Wade CE: Association of hemodilution and blood pressure in uncontrolled bleeding. *Journal of surgical research* 2013; 184(2):959–965.
37. Cheatham ML, Block EF, Promes J, Smith H, Dent D, Mueller D: Shock: an overview. *Irwin RS si Rippe JM (editori) "Intensive care medicine" Lippincott Williams & Wilkins, Philadelphia* 2008:1831–1842.
38. Sternberg EM: Neural-immune interactions in health and disease. *The Journal of clinical investigation* 1997; 100(11):2641–2647.
39. Andersson U, Tracey KJ: Reflex principles of immunological homeostasis. *Annual review of immunology* 2012; 30:313–335.
40. Hall S, Kumaria A, Belli A: The role of vagus nerve overactivity in the increased incidence of pneumonia following traumatic brain injury. *British journal of neurosurgery* 2014; 28(2):181–186.
41. Parrish WR, Rosas-Ballina M, Gallowitsch-Puerta M, Ochani M, Ochani K, Yang LH, Hudson L, Lin X, Patel N, Johnson SM *et al*: Modulation of TNF release by choline requires alpha7 subunit nicotinic acetylcholine receptor-mediated signaling. *Molecular medicine (Cambridge, Mass)* 2008; 14(9–10):567–574.
42. Fuentes JM, Hanly EJ, Aurora AR, De Maio A, Talamini MA: Anesthesia-specific protection from endotoxic shock is not mediated through the vagus nerve. *Surgery* 2005; 138(4):766–771.

Tables

Table 1. The comparison of arterial blood gas parameters at baseline and at the end of the experiments.

	Sham	Vag	HS	Vag+HS
pHa-1	7.46 ± 0.009	7.46 ± 0.02	7.46 ± 0.015	7.47 ± 0.012
PaCO₂-1	34.78 ± 1.24	32.95 ± 0.89	32.14 ± 1.59	33.25 ± 0.65
PaO₂-1	68 ± 1.12	68.66 ± 3.84	62.66 ± 1.6	65 ± 2.12
HCO₃⁻a-1	24.75 ± 0.50	23.55 ± 0.86	24.42 ± 0.73	24.4 ± 0.41
BEa-1	1.2 ± 0.34	0.12 ± 1	1.56 ± 0.45	1.23 ± 0.59
pHa-2	7.48 ± 0.007	7.48 ± 0.008	7.52 ± 0.013	7.51 ± 0.009
PaCO₂-2	30.17 ± 0.91	30.72 ± 1.28	26.46 ± 0.87	27.48 ± 1.19
PaO₂-2	73.24 ± 1.89	68.75 ± 1.25	67.83 ± 1.5	66 ± 1.82 *
HCO₃⁻-2	23.07 ± 0.36	22.86 ± 0.65	22.68 ± 0.35	22.24 ± 0.68
BEa-2	0.5 ± 0.24	0.12 ± 0.45	1.38 ± 0.42	0.51 ± 0.52
pHa-1	7.46 ± 0.009	7.46 ± 0.02	7.46 ± 0.015	7.47 ± 0.012

Data are mean±SE in the Sham (n=5), Vag (n=5), HS (n=7) and Vag+HS (n=7) groups. * (P<0.05), vs. the sham group; pHa-1, PaCO₂-1(mm Hg), PaO₂-1(mm Hg), HCO₃-1 (mmol/L) and base excess (BEa-1) are the arterial blood gas parameters at baseline. pHa-2, PaCO₂-2, PaO₂-2, HCO₃-2 and BEa-2 are the arterial blood gas parameters at the end of the experiments. PaO₂: arterial oxygen pressure, PaCO₂: arterial carbon dioxide pressure. Barometric pressure where the experiments were performed: 630 mm Hg

Table 2. The comparison of venous blood gas parameters at baseline and end of the experiments.

	Sham	Vag	HS	Vag+HS
pHv-1	7.43 ± 0.013	7.40 ± 0.02	7.43 ± 0.013	7.43 ± 0.009
PvCO ₂ -1	40.8 ± 0.99	39.9 ± 0.62	36.58 ± 2.12	37.25 ± 1.4
PvO ₂ -1	41.71 ± 0.64	42 ± 2	39.33 ± 1.8	40.4 ± 1.2
HCO ₃ ⁻ v-1	26.85 ± 0.44	25 ± 1.42	25.08 ± 0.66	25.1 ± 0.58
BEv-1	2.08 ± 0.32	0.06 ± 1.62	1.28 ± 0.32	0.93 ± 0.42
pHv-2	7.43 ± 0.005	7.44 ± 0.009	7.45 ± 0.009	7.44 ± 0.014
PvCO ₂ -2	37.92 ± 0.73	36.26 ± 0.87	34.85 ± 1.81	35.73 ± 1.32
PvO ₂ -2	36.85 ± 1.45	36.25 ± 1.6	31 ± 2.16	27.34 ± 2.13 ^{**#}
HCO ₃ ⁻ v-2	25.77 ± 0.37	25.1 ± 0.48	24.9 ± 0.82	24.25 ± 0.61
BEv-2	1.26 ± 0.18	1.16 ± 0.47	1.35 ± 0.51	0.7 ± 0.59

Data are mean±SE in the Sham (n=5), Vag (n=5), HS (n=7) and Vag+HS (n=7) groups. ** (P<0.01), vs. the sham group; # (p<0.05), vs. the Vag group. pHv-1, PvCO₂-1(mmHg), PvO₂-1 (mmHg), HCO₃⁻-1 (mmol/L) and base excess (BEv-1) are the venous blood gas parameters at baseline. pHv-2, PvCO₂-2, PvO₂-2, And HCO₃⁻-2 and BEv-2 are the venous blood gas parameters at the end of the experiments. PvO₂: venous oxygen pressure, PvCO₂: venous carbon dioxide pressure. Barometric pressure where the experiments were performed: 630 mm Hg.

Table 3. The comparison between the hemoglobin and arterial oxygen pressure and contents in the experimental groups.

Data are mean±SE

	Sham	Vag	HS	Vag+HS
Hb (g/dl)	13.69 ± 0.72	14.7 ± 0.69	11.82 ± 0.53	11.19 ± 0.49 #
PaO ₂ -2 (mmHg)	73.24 ± 1.89	68.75 ± 1.25	67.83 ± 1.5	66 ± 1.82 *
CaO ₂ -2 (ml/100ml blood)	17.82 ± 1.03	18.61 ± 0.82	14.98 ± 0.71	14.09 ± 0.53 *#
PvO ₂ -2 (mmHg)	36.85 ± 1.45	36.25 ± 1.6	31 ± 2.16	27.34 ± 2.13 **#
CvO ₂ -2 (ml/100ml blood)	13.05 ± 0.68	13.95 ± 0.82	9.02 ± 0.82 *#	7.06 ± 1.19 **##
PaO ₂ -PvO ₂ -2 (mmHg)	36.38 ± 2.78	32.5 ± 0.95	37.4 ± 3.78	39.9 ± 3.60
CaO ₂ -CvO ₂ -2 (ml/100ml blood)	4.76 ± 0.60	4.66 ± 0.55	5.95 ± 1.02	7.57 ± 0.66
PaO ₂ /FIO ₂ ratio	348.77 ± 9.01	327.38 ± 5.95	323.01 ± 7.41	314.76 ± 8.69 *

in the Sham (n=5), Vag (n=5), HS (n=7) and Vag+HS (n=7) groups. Hb: Hemoglobin concentration, PaO₂-2: arterial oxygen pressure, PvO₂-2: venous oxygen pressure, CaO₂-2: arterial oxygen content and CvO₂-2: venous oxygen content, PaO₂-PvO₂-2: arterial-venous oxygen pressure difference, CaO₂-CvO₂-2: arterial-venous oxygen content difference FIO₂: fractional concentration of oxygen. * (P<0.05) and ** (P<0.01), vs. the sham group; # (p<0.05), ## (p<0.05), vs. the Vag group. Barometric pressure where the experiments were performed: 630 mm Hg.

Figures

Figure 1

The real traces of blood pressure (BP) and heart rate (HR) recorded by a Powerlab system in the Sham (A), Vag (B), HS (C) and Vag+HS (D) groups. SS: Steady State; In-Com: initial Compensatory phase; De-Com: Decompensatory phase; Re-Com: Recompensatory phase; VBG: Venous blood gas; ABG: Arterial blood gas.

Figure 2

The Mean arterial blood pressure (MAP, E) and heart rate (HR, F) in the Sham (n=5), Vag (n=5), HS (n=7) and Vag+HS (n=7) groups. Data are mean±SE. *(P<0.05) and ** (P<0.01), vs. the baselines; # (p<0.05)

and ## ($p<0.01$), vs. the Sham group; a ($p<0.05$) and aa ($p<0.01$), vs. the Vag group; b ($p<0.05$), vs. the HS group; c ($p<0.05$), vs. the Vag+HS group.

Figure 3

The frequency domain components of HRV including VLF (A), LF (B), HF (C) and LF/HF ratio in the Sham ($n=5$), Vag ($n=5$), HS ($n=7$) and Vag+HS ($n=7$) groups. Data are mean $\pm$ SE. * ($P<0.05$) and ** ($P<0.01$), vs. the baselines; # ($p<0.05$) and ## ($p<0.01$, vs. the Sham group; a ($p<0.05$) and aa ($p<0.01$), vs. the Vag group; b ($p<0.05$) and bb ($p<0.01$), vs. the HS group; c ($p<0.05$) and cc ($p<0.01$, vs. the Vag+HS group.

Figure 4

The hematoxylin-eosin staining with magnification of 100X (A) and vascular congestion (B) in the lungs of the Sham ($n=5$), Vag ($n=5$), HS ($n=7$) and Vag+HS ($n=7$) groups. Data are mean $\pm$ SE. # ($p<0.05$), vs. the Sham group; a ($p<0.05$), vs. the Vag group; b ($p<0.05$), vs. the HS group.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [ARRIVEchecklistketabchi17.10.2019.docx](#)