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# Correlation of ketone bodies in blood and spleen

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**Abstract:** Starvation, diabetes, alcoholism and hypothermia cause ketoacidosis in the human body; therefore, the cause of death can be determined by analyzing ketone bodies in the blood of the deceased. In the case of decomposition of the cadaver, however, since collecting intact blood is impossible, ketone body analysis is performed using the spleen. However, the index for diagnosing ketoacidosis is based on blood concentration, and its correlation with ketone bodies present in the spleen remains unknown. In particular, since decomposition proceeds rapidly during summer, when temperature and humidity are high, understanding the correlation between ketone bodies in the blood and spleen is important to estimate the state at the time of death from a decaying body. Therefore, in the present study, the correlation between ketone bodies in the blood and spleen of the deceased was explored. Ketone bodies (beta-hydroxybutyric acid [BHB] and acetone) in the blood and spleen were analyzed and compared from autopsies (>100 $mg \cdot L^{-1}$ BHB, blood basis) conducted at the Daejeon Forensic Research Institute from June to December 2021. Moreover, the concentration of ketone bodies in the spleen juice and tissues was compared assuming the scenario of extreme decomposition. Ketone retention concentration in the blood and spleen was positively correlated, and the ratio of BHB concentration in the spleen to BHB concentration in the blood ranged from 0.52 to 1.08 (mean = 0.85 ± 0.12), although the ratio may vary depending on the degree of decomposition of the corpse.

**Key words:** ketoacidosis, blood, spleen, beta-hydroxybutyric acid, acetone, postmortem case

## 1. Introduction

In Korea, in the case of a fatal accident (disease and death due to external factors[1]), an autopsy is performed only when the cause of death must be estimated. In the case of death due to sudden diseases, such as heart disease, cerebrovascular disease, and diabetes, or in the case of an unknown cause of death, such as solitary death, the cause of death cannot be confirmed at the scene; therefore, chemical analysis of specimens along with autopsy is essential.

In chemical analysis of samples, certain indicators are used to estimate the cause of death. For instance, starvation, diabetes, and alcoholism cause ketoacidosis

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in the human body, and these conditions are diagnosed based on the analysis of ketone bodies in the blood of the deceased. Ketone bodies are substances produced in the liver through the oxidation of fatty acids and are formed by three components: acetone, acetoacetic acid, and beta-hydroxybutyric acid (BHB).[2] Acetyl coenzyme A (CoA), synthesized through the oxidation of fatty acids in the liver, enters the citric acid or Krebs cycle to generate adenosine triphosphate (ATP) or is converted to ketone bodies and transported to other tissues.[2-4] In the case of starvation and diabetes, excess ketone bodies are produced through the depletion of citric acid cycle intermediate, promoting the reaction of fatty acids in a direction that enhances ketone body production. In the case of alcoholism, nicotinamide adenine dinucleotide (NAD), which is used in alcohol metabolism, generates NADH (reduced form of NAD) and promotes conversion to BHB via the reduction of excess NADH.[2] Among ketone bodies, acetoacetic acid is produced first, BHB is produced via the reduction of NADH, and acetone is produced as a by-product via non-enzymatic decarboxylation of acetoacetic acid. Since ketone bodies produced through this pathway are strongly acidic, when ketone body levels in the blood increase, pH of the body fluid becomes acidic, leading to ketoacidosis.[3,5-7] BHB is used to discriminate ketoacidosis among ketone bodies.[8-10] BHB concentration related in ketoacidosis in detail in *Table* 1. In general, the analysis of ketone bodies in the blood of the deceased is based on the concentration of BHB(acetone concentration is used for identification along with BHB concentration), with values exceeding 250 $mg \cdot L^{-1}$ (BHB, dead body) suggestive of levels that can lead to ketoacidosis or death.[11]

When a corpse is discovered long after the time of death, it may not be possible to collect intact blood due to putrefaction. Putrefaction starts 2-3 hours after death at warm temperatures or 4-6 hours after death at cool temperatures.[12-14] Putrefaction is affected by temperature and humidity, with higher levels of each leading to a faster rate of decay.[12] In cases where a decaying corpse is discovered, such as in solitary deaths, it can be difficult to perform an autopsy.

The spleen is a large lymphoid organ that helps immune function, filters bacteria and antigens, and removes old red blood cells.[15,16] Therefore, when blood cannot be obtained, starvation, diabetes, and chronic alcoholism are estimated by analyzing ketone bodies in the spleen. However, the indicator for diagnosing ketoacidosis is blood concentration, and its correlation with ketone bodies in the spleen is not well-established. Since decomposition proceeds rapidly during summer, when temperature and humidity are high, understanding the correlation between ketone bodies in the blood and spleen is crucial to estimate the state at the time of death from a decaying body.

In the present study, the correlation between ketone bodies in blood and spleen of the deceased was explored and the correlation coefficient was calculated. Ketone bodies (BHB and acetone) in the blood and spleen were analyzed and compared from autopsies with blood BHB levels (>100 $mg \cdot L^{-1}$) conducted at the Daejeon Forensic Research Institute from June to December 2021. In addition, the concentration of ketone bodies in the spleen juice and tissue was compared assuming the scenario of extreme decomposition.

## 2. Materials and Methods

### 2.1. Sample treatment

Concentrations of acetone and BHB in the blood and spleen of 25 cases (>100 $mg \cdot L^{-1}$ BHB, based on

*Table* 1. Guide line for BHB concentration related in ketosis

| Value | Discussion |
|---|---|
| < 0.6 mmol/L | A normal blood ketone value |
| 0.6 to 1.5 mmol/L | Indicates that more ketones are being produced than normal, test again |
| 1.6 to 3.0 mmol/L | A high level of ketone and could present a risk of ketoacidosis |
| > 3.0 mmol/L | A dangerous level of ketones |

blood) of autopsies conducted at Daejeon Forensic Research Institute from June to December 2021 (IRB No. 906)-211116-BR-002-02) were compared.

When collecting a sample from the spleen, the spleen juice is extracted by compressing the spleen for analysis. BHB analysis was performed using the following method. After transferring approximately 300 μL of each blood or spleen juice into a 15 mL conical tube, 150 μL of a 350 $mg \cdot L^{-1}$ solution (in DI water) of internal standard [sodium DL-3-hydroxybutyrate (D4), CDN isotope] was added, and the mixture was gently stirred. After adding 150 μL of 0.05 M sulfuric acid (Sigma Aldrich) solution, 3 mL of ethyl acetate (EA) (Sigma Aldrich, extra pure) was added, and the mixture was shaken for 20 min at 40 rpm on a rotary shaker. After centrifuging the stirred sample at 4,000 rpm for 10 min, 2 mL of the supernatant was transferred to a glass vial and was nitrogen ($N_2$) concentrated in a water bath at 40 °C. Thereafter, 150 μL of a derivatization reagent [N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA); 1 % trimethyl chlorosilane] (Sigma Aldrich) was added to the glass vial. With the lid closed, the solution was allowed to react in an oven at 100 °C for 20 minutes. After the reaction completed, the mixture was cooled to room temperature (25 ºC) and then transferred to a vial for GC-MS analysis. The experiment was repeated three times for each sample, and the average value was applied. After adding the DL-beta-hydroxybutyric acid (Sigma Aldrich, extra pure, as standard material) by each concentration (20, 50, 80, 160, 200, 400, 500, 1000, 1500, 2000 mg/L as final concentration) to the glass vial, and it was completely nitrogen ($N_2$) concentrated in a water bath at 40 °C. The 300 μL of control blood was added to glass vial and the sample was pretreated following the same procedure as described above.

To compare the concentration of the spleen tissue and spleen juice, the samples were prepared as follows. The spleen juice was prepared by compressing the spleen to obtain liquid. The spleen tissue was finely chopped with scissors to make it as homogeneous as possible. Approximately 300 mg of spleen tissue and spleen juice were prepared, and the subsequent process was the same as the process described earlier.

Acetone analysis was performed as follows. In a 10 mL glass vial, 200 μL of saturated sodium chloride (NaCl) solution and 200 μL of 0.05 % tertiary butanol which is an internal standard (Thermo Fisher Scientific) were added, and the mixture was gently shaken to homogenize. After transferring 200 μL of blood or spleen juice into a glass vial, the vial was sealed with a rubber stopper and an aluminum cap. After adding the acetone standard for each concentration unit, the sample was prepared following the same procedure as described above.

### 2.2. GC-MS and GC-FID analysis

GC-MS (GC5977B, Agilent) was used for BHB analysis. The analytical conditions are summarized in *Table* 2. The DB-5MS capillary column was used as the GC column for analysis, and helium gas was used as the carrier gas at the flow rate of 1 $mL \cdot min^{-1}$. After holding at the initial column temperature of 100 °C for 8 min, the temperature was raised to 180 °C at the rate of 20 °C per min; then, after holding at 180 °C for 6 min, the temperature was raised to 280 °C

*Table* 2. GC/MS analytical conditions

| Item | Conditions |
|---|---|
| Column | DB-5MS caillary column |
| Column oven | 100 ºC (8 min) -20 ºC/min-180 ºC (6 min)-20 ºC/min-280 ºC (16 min) |
| Injection temperature/source temperature | 250 ºC / 250 ºC |
| Carrier gas flow | He gas, 1mL/min |
| Split ratio | 5:1 |
| Injection volume | 2 μL |
| Scan mode | Mass 50~550, scan speed 1.562(u/s), step size 0.1m/z |
| Target ion (for quantitative analysis) | 233(BHB), 237(internal standard) |

*Table* 3. GC-FID analytical conditions

| Item | Conditions |
|---|---|
| Column | DB-5 |
| Inlet temperature | 200 °C |
| Detector temperature | 250 °C |
| Sample equilibration time | 7 min |
| GC cycle | 4.5 min |
| Carrier gas | He |
| Split ratio | 5:1 |

at the rate of 20 °C per min and then allowed to stay at the final temperature for 16 min. Electron ionization (EI) was used as the ionization method in the mass spectrometer, and the temperature of the ion source was 250 °C. For qualitative and quantitative analysis, 233 *m/z* for BHB and 237 *m/z* for ISTD were used as the target ions. The 233 *m/z* is for identifying the derivatized BHB, and 237 *m/z* is for identifying the derivatized internal standard.

Samples prepared for acetone analysis were analyzed using GC-FID (7697A Headspace Autosampler, 7890B GC, Agilent). The analytical conditions are summarized in *Table* 3.

## 3. Results and Discussion

### 3.1. Correlation of ketone bodies in the blood and spleen

Of the 25 cases, 18 were ketoacidosis positive (250 $mg{\cdot}L^{-1}$ or higher BHB), and the BHB concentration ranged between 253 and 1,481 $mg{\cdot}L^{-1}$ (37 to 272 $mg{\cdot}L^{-1}$ acetone, blood basis). The remaining seven cases were ketoacidosis negative, with BHB concentration ranging from 100 to 232 $mg{\cdot}L^{-1}$ (<10 to 26 $mg{\cdot}L^{-1}$ acetone, based blood basis) (*Fig.* 1, *Table* 4) (The linearity of the calibration curve achieved an $R^2$ value of 0.9999 and standard curve was not shown.)

The deceased that tested positive for ketoacidosis suffered from chronic alcoholism and diabetes during their lifetime, suggesting that alcoholism and diabetes are correlated with excessive ketone body production. Of the 25 cases, 8 were men suspected of alcoholism or diabetes, and 3 were women (suspected of alcoholism or diabetes). If there was an underlying

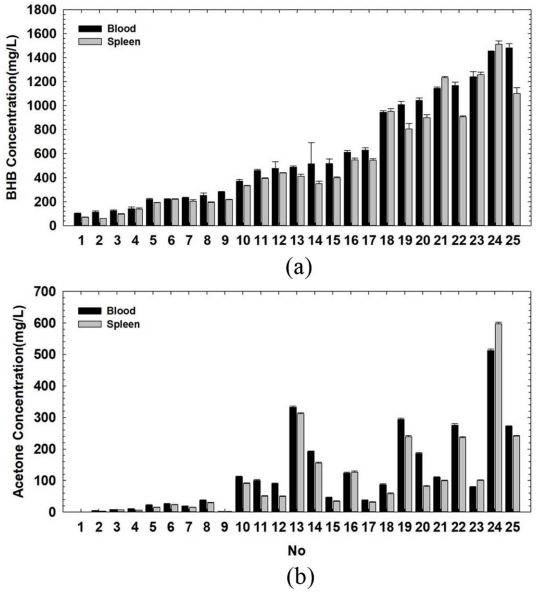


*Fig.* 1. The concentration of ketone bodies: (a) BHB and (b) Acetone in blood and spleen.

disease but the person (or the bereaved family) was not aware of or if it was a solitary death, unidentified underlying diseases may have existed.

BHB concentration was higher than acetone concentration in the study subjects (blood and spleen juice), and BHB and acetone concentrations were positively correlated. The quantitative correlation between BHB and acetone concentration was like reported correlation in diabetes and alcoholism-related deaths[17,18]; this may be attributed to the reversible production process of acetoacetic acid and BHB and the production ratio between acetone produced as a by-product of acetoacetic acid. However, the ratio of BHB and acetone concentrations was in the range of 0.01 to 0.68 (acetone/BHB concentration ratio, blood), and a constant correlation ratio was not observed. Obtaining a consistent index is considered difficult because various factors, such as sex, physical condition, health status, ketone body production mechanism, and distribution ratio of substances generated by metabolism, act in a complex manner. For example, in some cases, an excess amount of BHB and a low concentration of acetone can be observed,[19] and excess acetone is present in the blood during death due to diabetes,[20]

*Table* 4. BHB, acetone concentration and ratio of ketone bodies

| Case no. | Gender | Underlying disease | Type | BHB (mg/L) | Acetone (mg/L) | Ratio of BHB | Ratio of Acetone |
|---|---|---|---|---|---|---|---|
| 1 | M | None reported | Blood | 100 | <10 | 0.71 | 1.00 |
| | | | Spleen | 71 | <10 | | |
| 2 | M | None reported | Blood | 113 | 5 | 0.52 | 0.60 |
| | | | Spleen | 59 | 3 | | |
| 3 | F | None reported | Blood | 123 | 8 | 0.76 | 0.88 |
| | | | Spleen | 94 | 7 | | |
| 4 | M | None reported | Blood | 140 | 10 | 0.99 | 0.60 |
| | | | Spleen | 138 | 6 | | |
| 5 | F | Alcoholism, diabetes | Blood | 218 | 22 | 0.88 | 0.68 |
| | | | Spleen | 191 | 15 | | |
| 6 | M | Alcoholism | Blood | 222 | 26 | 0.99 | 0.88 |
| | | | Spleen | 219 | 23 | | |
| 7 | F | None reported | Blood | 232 | 19 | 0.88 | 0.79 |
| | | | Spleen | 203 | 15 | | |
| 8 | F | Alcoholism | Blood | 253 | 37 | 0.77 | 0.78 |
| | | | Spleen | 195 | 29 | | |
| 9 | M | Alcoholism | Blood | 282 | <10 | 0.77 | 1.00 |
| | | | Spleen | 216 | <10 | | |
| 10 | M | None reported | Blood | 371 | 112 | 0.89 | 0.81 |
| | | | Spleen | 332 | 91 | | |
| 11 | M | Alcoholism | Blood | 459 | 100 | 0.86 | 0.50 |
| | | | Spleen | 393 | 50 | | |
| 12 | M | High blood pressure | Blood | 478 | 90 | 0.92 | 0.54 |
| | | | Spleen | 439 | 49 | | |
| 13 | M | None reported | Blood | 488 | 333 | 0.85 | 0.94 |
| | | | Spleen | 413 | 312 | | |
| 14 | M | None reported | Blood | 515 | 192 | 0.68 | 0.81 |
| | | | Spleen | 350 | 155 | | |
| 15 | M | None reported | Blood | 518 | 46 | 0.77 | 0.74 |
| | | | Spleen | 400 | 34 | | |
| 16 | F | None reported | Blood | 611 | 124 | 0.90 | 1.02 |
| | | | Spleen | 548 | 127 | | |
| 17 | M | Alcoholism | Blood | 628 | 37 | 0.87 | 0.84 |
| | | | Spleen | 545 | 31 | | |
| 18 | M | Alcoholism | Blood | 945 | 87 | 1.01 | 0.67 |
| | | | Spleen | 952 | 58 | | |
| 19 | M | Alcoholism | Blood | 1008 | 294 | 0.80 | 0.81 |
| | | | Spleen | 807 | 239 | | |
| 20 | M | Alcoholism, diabetes | Blood | 1045 | 186 | 0.86 | 0.44 |
| | | | Spleen | 900 | 81 | | |
| 21 | F | diabetes | Blood | 1144 | 110 | 1.08 | 0.90 |
| | | | Spleen | 1233 | 99 | | |
| 22 | M | diabetes | Blood | 1169 | 276 | 0.78 | 0.86 |
| | | | Spleen | 906 | 236 | | |
| 23 | M | Alcoholism, diabetes | Blood | 1241 | 79 | 1.01 | 1.27 |
| | | | Spleen | 1258 | 100 | | |
| 24 | M | None reported | Blood | 1452 | 513 | 1.04 | 1.16 |
| | | | Spleen | 1511 | 597 | | |
| 25 | M | None reported | Blood | 1481 | 272 | 0.74 | 0.89 |
| | | | Spleen | 1101 | 241 | | |

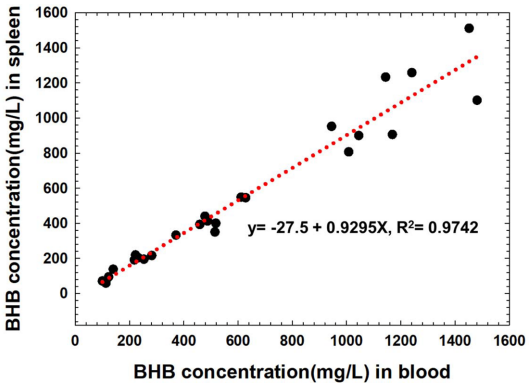


*Fig.* 2. The correlation of BHB concentration between in blood and spleen.

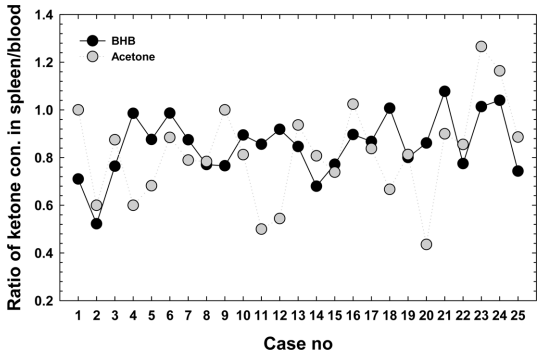


*Fig.* 3. Ratio distribution of ketone bodies (BHB and Acetone) concentration between in spleen and blood.

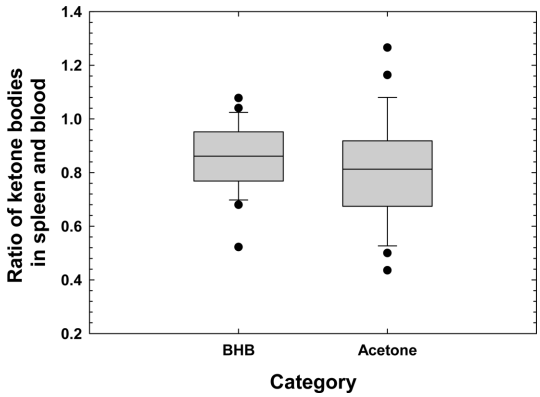


*Fig.* 4. Ratio of ketone bodies (BHB and Acetone) concentration between in spleen and blood: Average and standard deviation.

the correlation between BHB and acetone concentration may vary depending on the cause of death rather than converging at a constant value. Since furthermore, among the 25 cases studied here, 12 presented an underlying disease; therefore, determining the correlation between the actual cause of death and the concentration ratio was challenging.

The concentration of ketone bodies in the blood tended to be similar to or higher than that in the spleen, and this pattern was consistent regardless of the type of ketone body (*Fig.* 1, *Table* 4). BHB concentrations in the blood and spleen were positively correlated (*Fig.* 2). The correlation function of ketone bodies in the blood and spleen was estimated with a first-order regression line (y = -27.5 + 0.9295X), and the $R^2$ value was 0.9742. As calculated from the first-order regression line, when BHB concentration in the blood is approximately 150 mg·$L^{-1}$, BHB concentration in the spleen was approximately 120 mg·$L^{-1}$. The BHB concentration ratio in blood and spleen was 0.52 to 1.08 (mean = 0.85 ± 0.12, spleen /blood), and the acetone concentration ratio was 0.44 to 1.27 (mean = 0.82 ± 0.19, spleen/blood) (*Figs.* 3 and 4). The lower the concentration, the greater the standard deviation, because the concentration value produced a greater effect on the relative ratio. Meanwhile, the higher the indicated concentration value, the more similar the ratio (at ~0.8 level). The deviation of acetone concentration ratio in the blood and spleen was greater than that of BHB concentration ratio, and this may be because the concentration of acetone produced a greater effect on deviation as the value of acetone concentration was smaller (single-digit numbers). Many papers were reported that the concentration of BHB production in the liver is closely related to the concentration of BHB in the blood. Generally, hepatic BHB concentrations can show relatively higher levels than blood BHB concentrations. This is because of the difference between the amount of BHB produced and metabolized in the liver and the amount excreted into the blood during BHB production and metabolism processes in the liver.[21-23] In the experiment, it was confirmed that there is a positive correlation between the concentration of BHB in the spleen and blood, as in the liver and blood. However, it was found that the ratio of BHB concentration in blood and spleen varies depending on the degree of putrefaction.

In the 25 subjects of the present study, the concentration of ketone bodies in the blood was similar to or higher than that in the spleen. Therefore, when ketone body analysis is performed through spleen due to decomposition of the cadaver, the indicated concentration may be similar to or less than the blood concentration. If the ratio calculated from the results of the present study (~0.8) is applied, the positive standard of 250 mg·L$^{-1}$ BHB in blood may be indicated as a smaller value in the spleen; this should be considered when calculating the positive range in the future.

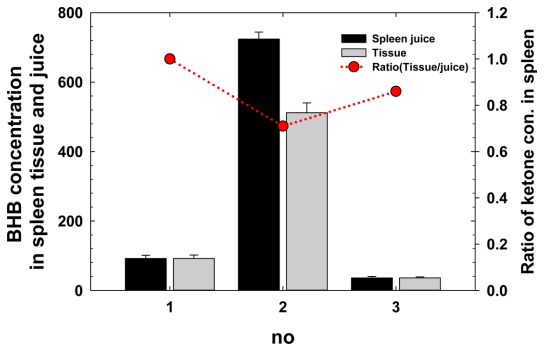


*Fig.* 5. BHB concentration and ratio in spleen tissue and juice.

### 3.2. Correlation of ketone bodies in the spleen juice and tissue

The spleen, which has not undergone decomposition, contains blood and is of the color of the blood. Moreover, collecting liquid samples is easy. However, as the decay progresses, the tissues turn dark brown, and collecting a liquid sample is difficult. At the start of decay, hemolysis occurs as the blood is degraded, and the surrounding tissue is infiltrated with hemoglobin, turning pale red. Decaying discoloration occurs throughout the body 24-36 hours after death, and the epidermis swells to form blisters.[14,24] After 3 to 5 days after death, the skin veins become reddish-brown to purple-greenish-brown, decay gas is formed in tissues and muscles, and the body goes through the mummification process.[12-14] In the case of cadavers that have undergone high degree of decomposition, even spleen fluid cannot be collected. Therefore, the concentration of ketone bodies may differ depending on the state of the specimen, such as spleen juice contained in blood, spleen juice that has undergone decomposition, and spleen tissue. To confirm this, the concentration of BHB was compared in the spleen juice and tissues that were not highly decomposed (for comparison between spleen juice and tissues, tissues in the normal state from which the spleen was not extracted by compressing the spleen were used).

The experiment was performed on three specimens, with one ketoacidosis-positive case (>250 mg·L$^{-1}$ BHB), one case with 100 mg·kg$^{-1}$ BHB, and one case with ≤50 mg·kg$^{-1}$ BHB. As shown in *Fig.* 5, BHB concentration in the spleen juice was higher than that in the tissue. In the positive standard sample, BHB concentration in the spleen juice was 724 mg·kg$^{-1}$ and that in the spleen tissue was 512 mg·kg$^{-1}$, which is nearly 70 % lower. In the ≤100 mg·kg$^{-1}$ concentration group, the value was in the range of 0.86 to 1.0 mg·kg$^{-1}$. Since the ratio of blood and tissue differs in the same amount of sample, the indicated BHB concentration may vary. Therefore, the ketone body concentration being reported may be significantly lower than that at the time of death in highly decomposed cadavers from which only dry spleen tissue can be collected.

### 3.3. The degree of putrefaction and the concentration of BHB

The BHB concentration ratio may vary depending on the degree of decay of the cadaver. A similar concentration of ketone bodies was detected in the blood and spleen of cadavers that had not undergone decomposition. This may be because intact blood can be collected even from the spleen. As decomposition progresses, the state of blood and tissue is altered, and the state of blood differs from that of specimens (red to dark-red tissues) collected from the spleen tissue. As the decay progresses, the state goes from intact blood to spleen juice and then to spleen tissue alone. From decomposed spleen tissue, the samples are obtained by squeezing the tissue; thus, the ratio of BHB present in the blood decreases as the ratio of components of spleen tissue increases In fact, when the BHB concentration ratio was close to 1, the

deceased was taken to the hospital by 119 paramedics while still alive, but died subsequently. Events with the ratio of 0.8 were recorded as being at the early stages of decay, while those with the ratio of 0.7 were recorded as decaying. In actual cases, when ketone bodies are analyzed in spleen samples, the reported concentration may be lower than the value in the blood at the time of death. This is because, during autopsy, spleen is used only when blood cannot be completely collected, such as from decomposed cadavers (*Table* 4).

However, the current study has limitations due to the restricted experiments performed on cases in which an autopsy was conducted, and the results were obtained without controlling for the degree of putrefaction of the actual corpse, the surrounding environment, and the personal characteristics of the corpse. Even in cases from which blood and spleens could be obtained simultaneously, the time of death and time of discovery may differ, and it is difficult to accurately predict the environment (such as temperature and humidity) in which the putrefaction of the dead body began. This is because of the complexity of biological samples, such as sex, age, health status, and underlying disease. Therefore, changes in the concentration ratio of ketone bodies in the blood and spleen according to the degree of decay must be explored.

Putrefaction of the corpse is related to several factors, and each variable warrants consideration. The rate of decay is driven by intrinsic and extrinsic factors. Intrinsic factors include age (the younger the body, such as in the fetal or neonatal stage, the slower the putrefaction), constitution (the decomposition rate is faster in obese people because of greater tissue mass, which is conducive to the development and spread of bacteria), and cause of death (diabetes and sepsis, among others).[25,26] Among the extrinsic factors, the most important variable is temperature. Putrefaction hardly occurs when the temperature is below 5 ºC, and putrefaction proceeds actively from 10 ºC. Temperatures in the range of 25-35 ºC are optimal for bacterial development.[25,27] In addition, biological factors, such as maggots and insects, are the second most influential variables following temperature. In the case death in an environment where the penetration of organisms, such as insects or flies, is easy, the putrefaction proceeds faster than in the case of death in a completely sterilized place.[28] Finally, humidity is another important variable. Higher the humidity, faster the putrefaction and slower the drying[29]; moreover, the better the ventilation, the faster the putrefaction.[25]

Selection of a biomarker that can estimate the postmortem interval (PMI) must be considered. It is very difficult to unambiguously quantify the degree of putrefaction of the deceased, and all methods currently used to estimate PMI remain inaccurate.[30-32] Among these, the catabolic activity of key enzymes on proteins, lipids, carbohydrates, and nucleic acids and the rate of this process may be used to estimate PMI.[30-32] In addition, leaching of electrolytes and blood pH changes due to accumulation of several ions and metabolites, such as bicarbonates, carbon dioxide, hydrogen ions, lactate, phosphoric, and formic acids, have been suggested as potential targets for PMI estimation.[36-40]

## 4. Conclusions

In the present study, some of the examined deceased who tested ketoacidosis positive suffered from chronic alcoholism and diabetes during their lifetime, suggesting that alcoholism and diabetes are correlated with excessive ketone body production. Moreover, the ratio of BHB concentration in the spleen to that in the blood ranged from 0.68 to 1.08 (mean = 0.87 ± 0.11, spleen/blood), which can be used as the positive criterion for suspected ketoacidosis. In addition, the concentration of ketone bodies in the spleen juice was higher than or similar to that in the spleen. In other words, the concentration of ketone bodies in the spleens was similar to or lower than that in the blood, and the ratio varied depending on the type of autopsy event. When it is necessary to determine the cause of death using spleen, it can be helpful in estimating the concentration of ketone bodies in the blood at the time of death.

Overall, determining the degree of putrefaction of

a biological sample is a challenging task; however, if there is a correlation between the concentration of ketone bodies in the blood and spleens according to the degree of putrefaction is established, it is thought that this would help estimate the blood concentration at the time of death based on the BHB concentration in the spleen.

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