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CORRELATION INTERACTION OF TOTAL BILE ACIDS WITH BASIC BLOOD BIOCHEMICAL INDICATORS IN MINKS (*Mustela vison* Schreber, 1777)

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Abstract

Liver diseases of various origins and complications arising from the gradual destruction of the bile ducts cause the accumulation of bile acids in the liver, bile, and blood serum. This induces a pro-inflammatory response and an increased production of reactive oxygen species, leading to cytotoxic effects. Any decrease in extraction efficiency caused by impaired liver function leads to an increase in the level of total bile acids in the blood serum. Serum or plasma bile acid levels are sensitive measures of liver function in all species, reflecting both hepatic synthesis, secretion, and reabsorptive function. Thus, testing of blood serum can reveal functional abnormalities of liver function before the formation of more pronounced clinical signs. This high sensitivity is very important for making a clinical diagnosis. The novelty of the research lies in the study of the correlation between the bile acids and other biochemical parameters associated with the functional state of the liver, which was carried out for the first time. The aim of the work was to identify the correlation between standard biochemical parameters and the content of bile acids in the blood serum, as well as to evaluate the bile acids in the blood serum as a predictor of the state of the hepatobiliary system. The experiments were carried out in 2022 at the Mermeriny fur farm (village Mermeriny, Kalinin District, Tver Province). Palomino minks (*Mustela vison* Schreber, 1777) were chosen as model animals. Blood sampling from 100 females and 100 males at the age of 1 year was performed by cutting the tip of the tail. The main criterion for the selection of minks is the absence of clinical signs of liver pathologies. The content of total protein, albumin, total bilirubin, alkaline phosphatase, glucose, cholesterol, total bile acids, de Ritis coefficient was determined on a biochemical analyzer URIT 8021A VET (RIT Medical Electronic Group Co., Ltd., China). Total bile acids were detected using the BSBE Bile Acid Kit (BSBE, China). As part of the scientific research work, a correlation was calculated (Spearman's rank correlation coefficient and correlation-regression analysis) between the classical predictors of the hepatobiliary system (total protein, albumin, total bilirubin, de Ritis coefficient, alkaline phosphatase, glucose, cholesterol) and general bile acids as the main studied quantity. It was revealed that the measurement of total bile acids in blood is a promising way to identify pathologies of the hepatobiliary system, especially those accompanied by a violation of protein and fat metabolism. This statement is supported by the stable correlation of the blood level of bile acids with such indicators as alkaline phosphatase and the de Ritis coefficient revealed during the study. The relationship between these indicators is consistent with the biochemical properties of these compounds. Bile acids can stimulate the synthesis of alkaline phosphatase, and the cytotoxic or cytoprotective function of various representatives of the bile acid pool directly affects the level of alanine aminotransferase and the de Ritis coefficient. The coincidence of values obtained using two methods of statistical analysis of correlations at a high confidence level ($P > 95$ %) indicates the reliable nature of the identified relationships. When conducting these tests, it is necessary to take into account the heterogeneity of the results depending on the sex of the animals. In males, a moderate positive relationship between bile acids and concentrations of cholesterol and albumin was the most obvious, $0.2 \geq r \leq 0.5$ as per correlation and regression analysis method and $0.3 \geq r \leq 0.5$ as per Spearman's rank correlation coefficient technique. In females, there was a strong positive correlation between blood levels of bile acids and the total protein and bilirubin concentrations, $0.7 \geq r \leq 0.9$ for both calculation methods used.

Keywords: bile acids, minks, hepatobiliary system, liver, biochemical parameters, correlation

Bile acids are a group of steroid compounds derived from cholesterol [1]. They have a unique stereochemistry, hydroxyl groups and an aliphatic side chain with a terminal carboxyl residue [2]. These molecules have historically been described as lipid solubilizing agents and pancreatic enzyme activators, concomitant with their role in intestinal absorption [3]. While bile acids are endogenously toxic at elevated concentrations due to their amphipathic structure, some authors [4-7] point out that they also have endocrine and metabolic functions that include self-regulation of their synthesis, transport, and detoxification. Bile acids are involved in energy-producing reactions, in lipid and glucose homeostasis and affect the composition of the intestinal microbiota [8].

Changes in the metabolism and transport of bile acids lead to pathological state [7]. For example, an increased amount of these compounds in the enterohepatic circulation system can cause pathologies of the liver and intestines [9]. Conversely, deficiency results in nutrient malabsorption and fat-soluble vitamin deficiencies [10]. Therefore, a balanced metabolism of bile acids is important due to their significant role in homeostasis.

Liver diseases of various origins and severity, resulting from the gradual destruction of the bile ducts, lead to the accumulation of bile acids in the liver, bile and blood [11]. This process induces an inflammatory response and increased production of reactive oxygen species [12], leading to cytotoxic effects [13].

One of the functions of the liver is the removal of bile acids from the portal blood circulation, which is provided by bile acid transporters located on the sinusoidal membrane of hepatocytes [14]. The high extraction efficiency determines the low content of total bile acids in the peripheral blood compared to the portal blood. Any decrease in extraction efficiency caused by impaired liver function leads to an increase in the content of total bile acids in the blood serum [15, 16]. The amount of bile acids in serum or plasma is determined by hepatic synthesis, secretion, and reabsorption [16]. Therefore, blood serum testing makes it possible to detect liver dysfunction before the development of more pronounced clinical signs, which is very important for clinical diagnosis [16].

In various hepatobiliary disorders, elevated concentrations of bile acids in the blood serum were found, and therefore they are currently considered as one of the predictors of the state of the hepatobiliary system [17]. However, sensitivity, specificity and predictive value of such diagnostics is not fully understood. It has been proven that this method is able to detect hepatobiliary disorders, but it has not been compared with classical diagnostic methods (primarily with biochemical methods) [18].

Here, our findings for the first time established a correlation between the content of total bile acids and classical (biochemical) indicators of the state of the hepatobiliary system.

Our goal was to reveal the relationship between standard biochemical parameters and the content of bile acids in the blood serum and to evaluate these parameters as predictors of the hepatobiliary status.

Materials and methods. The experiments were carried out at the Mermeriny fur farm (Mermeriny village, Kalininsky District, Tver' Province, 2022). Palomino minks (*Mustela vison* Schreber, 1777) were chosen as model animals [19].

Blood of 1-year old 100 females and 100 males was takes from an incision at the tip of the tail into improvacuter vacuum test tubes for biochemical research (Guangzhou Improve Medical Instruments Co., Ltd., China) with blood clotting activator [20]. All asepsis and antiseptic measures were observed. The main criterion for selecting minks was the absence of clinical manifestations of liver pathologies.

Total protein, albumin, total bilirubin, alkaline phosphatase, glucose, cholesterol, total bile acids, aspartate aminotransferase and alanine aminotransferase (with further calculation of the de Ritis coefficient) was measured (a URIT 8021A VET biochemical analyzer, URIT Medical Electronic Group Co., Ltd., China). The concentration of total protein was determined by the biuret method using a color reaction with copper sulfate (AO LenReaktiv, Russia) in an alkaline medium. Albumins were quantified colorimetrically with bromcresol green (AO LenReaktiv, Russia). Total bilirubin was measured colorimetrically by diazo method according to Jendrashik-Cleggorn-Grof with sodium nitrate (AO LenReaktiv, Russia). The de Ritis coefficient was calculated as the ratio of the activity of serum aspartate aminotransferase and alanine aminotransferase [21]. Glucose content was assessed by the standard glucose oxidant method (glucose oxidase manufactured by OOO Biopreparat, Russia), cholesterol content by the Ilka method (Ilka reagent manufactured by AO LenReaktiv, Russia) [21].

Total bile acids were detected using a BSBE bile acid kit (BSBE, China). The method is based on the chemical properties of bile acids, that is, in the presence of ThioNAD, 3-α-hydroxysteroid dehydrogenase (3α-HSD) converts bile acids into 3-ketosteroids and Thio-NADH. The reaction is reversible and 3α-HSD can again convert 3-ketosteroids and Thio-NADH to bile acids and Thio-NAD [21, 22]. With an excess of NADH, an enzymatic cycle occurs, and the rate of Thio-NADH production was determined by a specific change in optical density at $\lambda = 405$ nm [21, 22].

The results obtained were considered random variables, for the processing of which stochastic modeling (correlation-regression analysis) was used [23].

Correlation-regression analysis was carried out using Pearson's formula [25] regarding the indicator of total bile acids as diagnostic criteria with an unknown correlation with respect to other indicators:

$$r_{xy} = \frac{\sum_{t=1}^{m}(x_t - \bar{x})(y_t - \bar{y})}{\sqrt{\sum_{t=1}^{m}(x_t - \bar{x})^2 \sum_{t=1}^{m}(y_t - \bar{y})^2}} = \frac{\mathrm{cov}(x,y)}{\sqrt{s_x^2 s_y^2}},$$

where $\bar{x}, \bar{y}$ are sample means xx^m, y^m, s_x^2, s_y^2 are sample variances, $r_{xy} \in [-1, 1]$.

Based on the results of the analysis, in order to confirm the conclusions about the presence, magnitude and strength of the correlation, the Spearman rank correlation coefficient was additionally calculated due to the small sample size and the deliberate nonparametric nature of the studied parameters [24].

The main studied value was the content of total bile acids, the correlation coefficients of other parameters were calculated vs. this criterion using the formula:

$$p = 1 - 6\frac{\sum d^2}{n^3 - n},$$

where d^2 are the squared differences between the ranks, N is the number of traits that participated in the ranking.

The results were processed using the Statistica 6.0 program (StatSoft, Inc., USA). Mean values of indicators (*M*), standard errors of means (±SEM) were calculated, the correlations, their magnitude and strength were determined. The lack of reliability calculation was due to the exploratory character of the survey and the absence of control groups.

Results. The choice of the Palomino breed was due to the species propensity of these minks to hepatopathy [19] and the maximum rate of enterohepatic circulation of bile acids among mammals. In the examined animals, protein, fat, carbohydrate and pigment metabolism, which serve as indicators of the functional state of the liver, were characterized as a variant of the physiological norm, that is, the indicators did not go beyond the reference values.

Blood biochemical parameters of Palomino minks (*Mustela vison* Schreber, 1777) without clinical manifestations of liver pathologies (M±SEM, the Mermeriny fur farm (Mermeriny village, Kalininsky District, Tver' Province, 2022)

Parameter	Males (n = 100)	Females (n = 100)	Reference values
Total protein, g/l	76.68±2.14	80.09±1.02	50-81
Albumins, g/l	34.75±0.71	33.32±0.56	20.0-50.0
De Ritis coefficient (AsAT/AlAT)	0.92	0.86	0.85-1.75
Alkaline phosphatase, IU/l	66.45±2.31	76.88±4.12	25.58-147.69
Glucose, mmol/l	2.87±0.11	2.99±0.17	6.5-12.1
Cholesterol, mmol/l	5.90±0.10	6.94±0.23	3.7-7.02
Total bilirubin, μmol/l	5.09±0.32	5.37±0.43	3.42-26.06
Total bile acids, μmol/l	4.63±1.02	5.56±1.19	2.00-7.00

N o t e. AsAT — aspartate aminotransferase, AlAT — alanine aminotransferase.

Figure 1 shows the results of the correlation-regression analysis (weak correlations were not accounted).

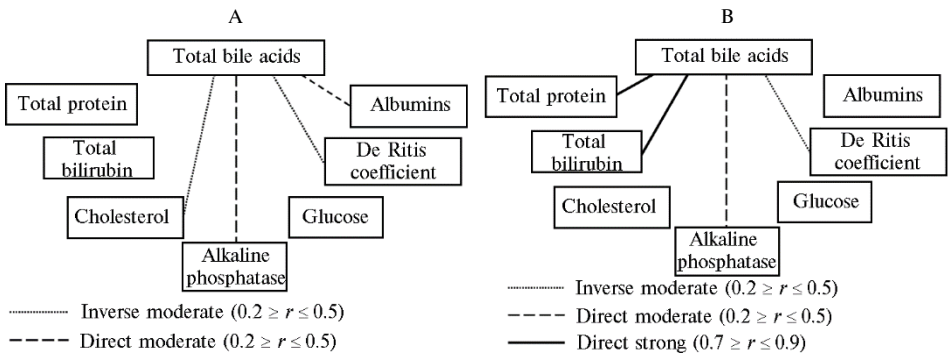

Fig. 1. Correlation-regression analysis of the relationship between the blood concentration of bile acids and the main blood biochemical parameters in Palomino mink (*Mustela vison* Schreber, 1777) males (A, n = 100) **and females** (B, n = 100) (P > 95%, the Mermeriny fur farm (Mermeriny village, Kalininsky District, Tver' Province, 2022).

In mink males, there was no correlation between the content of bile acids and the amount of total protein and bilirubin. (Fig. 1, A). This is due to the fact that the synthesis of proteins and bilirubin in the body is not associated with bile formation. Currently, it is believed that the concentration of bilirubin in the blood serum can be an individual constitutional indicator that goes beyond the reference values, which does not indicate pathological processes in the hepatobiliary system [26]. Thus, when studying the pathogenesis of hereditary constitutional hyperbilirubinemia, changes in the content of bile acids were not recorded [27].

A direct moderate correlation of the content of bile acids with albumins was due to the fact that the vast majority of them are synthesized by the liver, that is, there is a relationship between the protein-synthesizing and secretory functions of the organ. The direct moderate relationship with the activity of alkaline phosphatase and the inverse moderate relationship with the de Ritis coefficient can be explained by the biochemical properties of these compounds. Bile acids are able to stimulate the synthesis of alkaline phosphatase [28], and the cytotoxic or cytoprotective function of various members of the bile acid pool directly affects the content of alanine aminotransferase and the de Ritis coefficient. The amount of glucose in the blood serum, despite the fact that the main regulation of this indicator is carried out with the participation of the liver, depends on many neuroendocrine reactions, and therefore the lack of correlation with the content of bile acids is natural. The content of cholesterol as one of the precursors of bile acids had an inverse moderate correlation with their content.

The discrepancy in some correlation relationships between male and

female minks is explained by their physiological and hormonal differences (see Fig. 1). In particular, the high association with total protein and bilirubin, as well as the lack of association with albumin in females, were due to the estrus period [29, 30], during which the quantitative value of the first two indicators increased and the albumin content decreased. The lack of relationship with cholesterol content is explained by its predominant role in steroidogenesis in females (31).

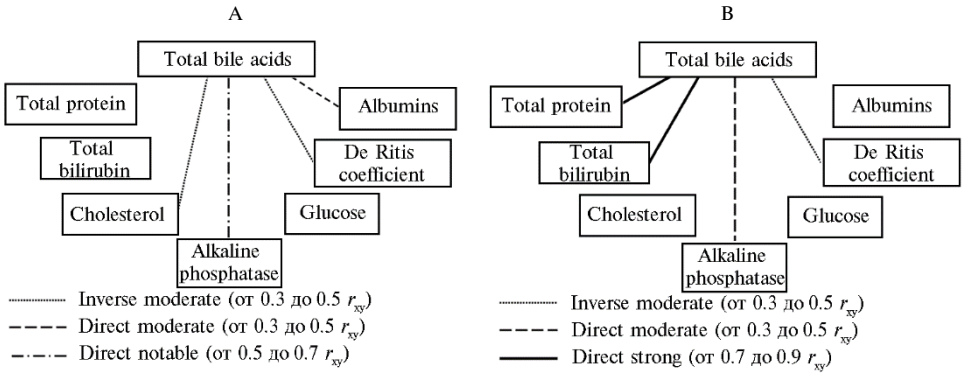

Fig. 2. Calculation of the Spearman rank correlation coefficient between the blood concentration of bile acids and the main blood biochemical parameters in Palomino mink (*Mustela vison* Schreber, 1777) males (A, $n = 100$) **and females** (B, $n = 100$) **minks (Mustela vison Schreber, 1777)** (P > 95%, the Mermeriny fur farm (Mermeriny village, Kalininsky District, Tver' Province, 2022).

To confirm the reliability of correlations, the Spearman rank correlation coefficient was additionally calculated (Fig. 2). The coincidence of the results of the correlation analysis performed by two methods indicates the reliable nature of the identified relationships. Theoretically, each factor that disrupts the enterohepatic circulation leads to a change in the content of bile acids in the blood serum. At that, we found a strong correlation between the amount of bile acids and the activity of alkaline phosphatase, as well as the de Ritis coefficient.

In the last few decades, the main predictor role of bile acids in blood serum has been associated not only with their total amount, but also with the qualitative composition of the pool, the components of which differ both in chemical activity and mechanisms of action from cytotoxicity to cytoprotection [17]. Nevertheless, the content of total bile acids remains an important prognostic and diagnostic criterion in the detection of hepatopathy of various origins.

Thus, the content of total bile acids in blood serum is a promising predictor of the hepatobiliary status, which can be used in diagnostics together with classical biochemical tests for total protein, albumin, total bilirubin, de Ritis coefficient, alkaline phosphatase, glucose, and cholesterol. Total bile acids may serve as indicators of hepatobiliary pathologies, especially those accompanied by a violation of protein and fat metabolism. This confirms the stable correlation relationship of this quantitative indicator with the activity of alkaline phosphatase and the de Ritis coefficient (for both sexes, a direct moderate correlation is $0.2 \geq r \leq 0.5$ according to correlation-regression analysis and $0.3 \geq r \leq 0.5$ when calculating the Spearman rank correlation coefficient. The coincidence of the values obtained using the two methods for statistical analysis of correlations at a high confidence level (P > 95%) indicates the significant character of the identified relationships. The character of some correlations varied depending on the sex of minks. Iin males, the most obvious relationship was traced between the blood bile acid concentration and cholesterol and albumins (direct moderate correlation $0.2 \geq r \leq 0.5$ according to correlation-regression analysis and $0.3 \geq r \leq 0.5$ when calculating the Spearman correlation coefficient). In females, there was correlation with total

protein and bilirubin level (direct high correlation $0.7 \geq r \leq 0.9$ for both calculation methods). We plan further studying the correlations between bile acids and biochemical parameters of metabolism in healthy animals and under various pathologies, given the predictor role of the bile acid pool qualitative composition.

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