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Case Control Study

Detection and Analysis of Serum Bile Acid Profile in Patients with Colonic Polyps

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Abstract

BACKGROUND

Analyzing the variations in serum bile acid (BA) profile can provide a certain biological basis for early warning and prevention of various diseases. There is currently no comprehensive study on the relationship between the serum BA profile and colonic polyps.

AIM

To study the serum BA profile detection results of patients with colonic polyps, and analyze the correlation between BA and colonic polyps.

METHODS

From January 1, 2022, to June 1, 2023, 204 patients with colonic polyps who were diagnosed and treated at Zhongda Hospital Southeast University were chosen as the study subjects, and 135 non-polyp people who underwent physical examination were chosen as the control group. Gathering all patients' clinical information, typical biochemical indicators, and BA profile.

RESULTS

Compared with the control group, the serum levels of taurocholic acid (TCA), glycocholic acid (GCA), glycochenodeoxycholic acid (GCDCA), and taurochenodeoxycholic acid (TCDCA) in the colonic polyp group were significantly higher than those in the control group, while the content of deoxycholic acid (DCA) was lower than that in the control group ($P<0.05$). When colonic polyps were analyzed as subgroups, it was shown that there was a strong correlation between changes in the BA profile and polyp diameter, location, morphology, pathological kind, *etc.*

CONCLUSION

The serum BA profile showed significant changes in patients with colonic polyps, with a significant increase in primary conjugated BA content and a decrease in secondary free bile acid DCA content. There is a certain correlation between primary free BA and pathological parameters of polyps.

INTRODUCTION

Colonic polyps are lesions that protrude from the mucosal surface into the large intestine lumen, and they can be further classified into adenomatous polyps and non-adenomatous polyps based on their pathology^[1]. The second-highest death rate of all malignancies is associated with colon cancer, which is the third most frequent malignancy worldwide^[2]. Colonic polyps are precancerous lesions of colonic cancer, especially adenomatous polyps. Over 50% of colonic cancer is derived from adenomas, which make up about two-thirds of colonic polyps^[3]. Early-stage colon cancer is typically found *via* a colonoscopy and does not typically present with any overt clinical symptoms. The incidence of colonic cancer can be decreased and the survival rate increased by early detection of precancerous lesions, early diagnosis, and early treatment. At present, the initial diagnosis of the disease mainly relies on endoscopic examination, further diagnosis requires pathological biopsy^[4]. Therefore, finding ways

to lessen them and enhancing the degree of non-invasive colonic polyp identification and treatment can help to some extent reduce the incidence of colonic cancer.

Bile acid (BA) is a major component of bile, synthesized by ³cholesterol in the liver and stored in the gallbladder. It is secreted into the small intestine after eating to promote the digestion and absorption of lipids and lipophilic vitamins^[5]. Meanwhile, as a cellular signaling molecule, BA also regulates biological processes by stimulating various signaling pathways, participating in the regulation of glucose metabolism, energy homeostasis, and immune response in the body. Analyzing the variations in serum BA profile can provide a certain biological basis for early warning and prevention of various diseases. There is currently no comprehensive study on the relationship between the serum bile acid profile and colonic polyps, despite the fact that numerous studies have demonstrated that high levels of total bile acid (TBA) are a risk factor for colonic cancer^[6]. In this study, the levels of 15 serum BA components were compared between patients with colonic polyps and healthy people. Additionally, alterations in the serum BA profile of patients with colonic polyps were initially explored, and the relationship between BA components and colonic polyps was analyzed.

MATERIALS AND METHODS

2.1 Research object.

204 individuals who were hospitalized and diagnosed with colonic polyps at Zhongda Hospital Southeast University between January 1, 2022, and June 1, 2023 were chosen as the colonic polyp group by reviewing the electronic medical record system. There were 114 men and 90 women in this group, with an average age of (57.19±9.43) years. Inclusion criteria: (a) Patients with pathological diagnosis of colonic polyps through colonoscopy, aged between 30 and 75 years old; (b) Routine biochemical tests and serum BA profile have been completed before undergoing colonoscopy. Exclusion criteria: (a) Previous history of inflammatory bowel disease; (b) Previous intestinal surgery (excluding appendectomy); (c) Previous liver and biliary

system diseases, such as viral liver disease, cirrhosis, autoimmune hepatitis, sclerosing cholangitis, etc; (d) Severe cardiopulmonary and renal dysfunction; (e) Patients with other malignant tumors; (f) Patients who have received chemotherapy or immunotherapy. The control group consisted of up of 135 healthy people who were examined by colonoscopy in our institution throughout the same time period but were not found to have any significant abnormalities. They had an average age of (55.35 ± 8.79) years, with 61 men and 74 women. The exclusion criteria are the same as those for the colonic polyp group. This study was approved by the Ethics Committee of Zhongda Hospital (2021ZDSYLL297-P01). Retrospective study without informed consent.

2.2 Research methods.

Gathering demographic data and clinical test results about the research subjects, such as age, gender, body mass index(BMI), alanine transaminase(ALT), aspartate transaminase(AST), total cholesterol(TC), and serum BA profile. Additionally, gathering the pathological characteristics of colonic polyps, including their number, size, location, and whether or not they have a pedicle. 15 different types of BA were identified in the BA profile using high-performance liquid chromatography tandem mass spectrometry (LC-MS/MS), including: (a) primary free BAs: cholic acid (CA), chenodeoxycholic acid (CDCA), (b) primary conjugated BAs: taurocholic acid (TCA), glycocholic acid (GCA), taurochenodeoxycholic acid (TCDCA), glycochenodeoxycholic acid (GCDCA), (c) secondary free BAs: deoxycholic acid (DCA), ursodeoxycholic acid (UDCA), lithocholic acid (LCA); (d) secondary conjugated BAs: tauroursodeoxycholic acid (TDCA), glycodeoxycholic acid (GDCA), tauroursodeoxycholic acid (TUDCA), glycoursodeoxycholic acid(GUDCA), tauroolithocholic acid(TLCA), and glycolithocholic acid (GLCA).

2.3 Statistical analysis.

Statistical analysis was conducted using SPSS 26.0 software. The normality test of the data was conducted using the Kolmogorov-Smirnon test. The measurement data of normal distribution was expressed by mean \pm standard deviation, and the comparison

between the two groups is conducted using independent sample *t*-test. The measurement data of skewed distribution were represented by median and interquartile spacing [M (P25, P75)]. The independent sample non parametric Mann Whitney *U* test is used for comparison between the two groups, and the Kruskal-Wallis *H* rank sum test is used for comparison between multiple groups. Chi-square test was used for counting data between groups. The risk factors for colon polyps were analyzed using univariate and multivariate logistic regression analysis, and the results were expressed using Odds Ratio (OR) and 95% Confidence Interval (95%CI). *P*-values < 0.05 were considered statistically significant. Using the Metabianalyst platform to draw heat maps, perform orthogonal partial least squares discriminant analysis (OPLS-DA), and calculate the variable importance in projection (VIP) of predicted variables; And combined with SPSS 26.0 software for analysis, differential bile acid components were screened under conditions of *P*<0.05 and VIP>1.

RESULTS

3.1 Comparison of research subjects' overall situations

In this retrospective analysis, 204 people made up the colonic polyp group and 135 people made up the control group. Age, BMI, gender, ALT, AST, and TC did not statistically differ between the two groups (*P*>0.05), demonstrating comparability (Table 1).

3.2 Comparison of serum TBA levels between colonic polyp group and control group

The TBA content did not differ statistically significantly between the colonic polyp group and the control group, according to an analysis of the 15 different forms of BA present in the serum of the two groups (Colonic polyp group: 2990.100(1384.950, 5489.750), Control group: 2490.500(1337.300, 4519.400), *P*=0.138).

3.3 Comparison of differences in serum BA composition between colonic polyp group and control group

The results of two sets of BA profile detection are shown in Table 2. Using the OPLS-DA model to search for differential metabolites between the colonic polyp group and the control group, it can be observed from the score chart (Figure 1A) that the sample points of the two groups are relatively concentrated, and the differences between the data groups are not significant. To further screen for BA with discrepancies, use VIP values (Figure 1B). It is evident that the two groups' BAs differ in the following ways: GDCA, DCA, GCA, GCDCA, TCA, TCDCA (VIP>1). TBA, DCA, GCA, GCDCA, TCA, and TCDCA were all statistically different ($P<0.05$) between the two groups, according to SPSS software analysis. While the concentration of DCA was lower than that of the control group, it was significantly greater than that of TBA, GCA, TCA, and TCDCA in the colonic polyp group. The other BA components (Table 2) showed no statistically significant change ($P>0.05$). Differential BA components GCA, GCDCA, TCA, TCDCA, and DCA were screened under the conditions of $P<0.05$ and VIP>1. Additionally, the heat map (Figure 2) can be used to reference the expression of BA profiles in distinct samples.

3.4 Analysis of the relationship between serum BA levels and clinical pathological parameters of colonic polyps

Colonic polyps can be classified using subgroup analysis in accordance with different pathological types, numbers, sizes, locations, and shapes (Table 3.1). Through subgroup analysis, we found that: (1) In terms of CA, CDCA, UDCA, and TUDCA, there was a statistically difference ($P<0.05$) between the adenomatous colonic group and the non-adenomatous polyp group. In comparison to the non-adenomatous polyp group, the CA, CDCA, UDCA, and TUDCA content in the adenomatous polyp group was lower (Table 3.2). (2) There is no statistical difference in the composition of BA between the single and multiple groups ($P>0.05$) (Table 3.3). (3) There was a statistical difference ($P<0.05$) between the two groups with polyp diameter<1cm and ≥ 1 cm in CA, CDCA, UDCA, GUDCA, and TUDCA, and the content of CA, CDCA, UDCA, GUDCA, and TUDCA in the group with polyp diameter ≥ 1 cm was higher than

that in the group with polyp diameter<1cm (Table 3.4). (4) There were statistical differences ($P<0.05$) among CA, CDCA, GCA, and GCDCA in the left colon group, right colon group, and total colon group (Table 3.5). Through pairwise analysis, it was found that there was a significant statistical difference in GCDCA between the left and right colon groups ($P=0.008$), and the GCDCA content in the right colon group was significantly higher than that in the left colon group; There was a significant statistical difference ($P=0.000$) between the left colon group and the whole colon group in terms of CA content. The content of CA in the left colon group was significantly higher than that in the whole colon group; There is a statistical difference between the right colon group and the whole colon group in terms of CA ($P=0.008$), GCA ($P=0.005$), and GCDCA ($P=0.015$). The content of CA, GCA, and GCDCA in the right colon group is significantly higher than that in the whole colon group. (5) There was a statistical difference ($P<0.05$) between the pedicle polyp group and the non-pedicle polyp group in terms of CA, CDCA, UDCA, and GUDCA. The content of CA, CDCA, UDCA, and GUDCA in the pedicle polyp group was significantly higher than that in the non-pedicle polyp group (Table 3.6). Therefore, we speculate that the changes in bile acid profile are closely related to polyp diameter, polyp site, polyp morphology, pathological type, etc.

3.5 Logistic regression model analysis of risk factors for colonic polyps

A univariate logistic regression analysis using the presence or absence of colonic polyps as the dependent variable and other indicators as the independent variables was carried out to evaluate the risk factors for colonic polyps. The results showed that CDCA ($B=0.000$, $OR=1.000$), GCDCA ($B=0.000$, $OR=1.000$), and primary BA ($B=0.000$, $OR=1.000$) were associated with the risk of colonic polyps and were risk factors for colonic polyps ($P<0.05$), as shown in Table 4. The results of multivariate logistic regression analysis using the statistically differences in the aforementioned univariate analysis indicators revealed that CDCA, GCDCA and primary BA were not independent risk factors for the development of colonic polyps ($P>0.05$).

DISCUSSION

This study found that compared with the control group, the serum primary conjugated BAs, TCA, GCA, GCDCA and TCDCA levels in the colonic polyp group¹ were significantly higher than those in the control group ($P < 0.05$), while the secondary free BAs, DCA content was lower than that in the control group. K ü hn T *et al*^[7] included 581 cases of primary colonic cancer diagnosed between 1993 and 2008, found that five primary conjugated BAs,⁵ GCA, TCA, GCDCA, TCDCA, and GHCA, as well as two secondary conjugated BAs, GDCA and TDCA were positively correlated with colonic cancer risk. Experts believed that an increase in primary conjugated BAs can promote the occurrence of colonic cancer, and the outcomes of this investigation supported those of our study. The concentration of fecal BA is the main subject of several relevant investigations. Sun L *et al*^[8] demonstrated that CDCA, DCA, and LCA increased in the feces of colon cancer patients whereas GCDCA decreased. By comparing the Alaskan aboriginals (AN) with the highest incidence rate of colonic cancer and the African rural people (RA) with the lowest incidence rate, Ocivirk S *et al*^[9] discovered that the detection rate of colonic polyps in the AN population was higher than that in the RA population, and the concentration of DCA, CA, and CDCA in the AN population's feces was also significantly higher than that of the RA sample. Kawano *et al*^[10] compared the concentration of BA in fecal samples from 366 patients who underwent endoscopic resection of colonic tumors (tumor group) and 24 control groups (control group) with no abnormalities in the large intestine, and followed up the tumor group. The findings revealed that while there was no change in CA levels between the two groups, the fecal DCA¹ levels in the tumor group were considerably greater than those in the control group. In the tumor group, the subgroup with high fecal DCA levels is more likely than the subgroup with low DCA levels to experience a recurrence of large adenomas (>3mm) after four years. On the basis of the aforementioned studies, we discovered that DCA may be linked to the development of colonic cancers, particularly when fecal DCA concentration rises and serum DCA concentration falls.

On the pattern of alterations in other BA components in colon cancer patients, there is yet no unified conclusion. The outcomes of various research' detection and analysis varies substantially. However, it is evident that colon cancer patients' serum BA profiles have changed from those of healthy people, and that these alterations in the BA spectrum are somewhat correlated with the formation and progression of colon cancer.

Previous studies have analyzed the role and mechanism of BA profile in the occurrence and development of colonic tumors. The commonly accepted theory holds that while increasing the concentration of UDCA may restrict the onset and development of cancers, increasing the concentration of DCA in the BA profile may promote the emergence of colonic malignancies ^[11,12]. In 1940, DCA was first proven to be a carcinogen capable of causing mouse colonic cancer ^[11]. It can induce excessive proliferation of colonic epithelium, disrupt cell membranes, promote excessive production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), cause oxidative stress, damage DNA, induce gene mutations, and NF- κ B activation by activating epidermal growth factor receptor (EGFR) and protein kinase C (PKC) leads to pathological changes in the tissue^[13]. The activation of NF- κ B in intestinal inflammation can induce the expression of cytokines to support inflammation related tissue damage, such as TNF- α , IL-6 and other chemokines. Therefore, NF- κ B may also promote the occurrence of colonic cancer by maintaining a continuous inflammatory process in the intestinal tissue^[14]. In addition, studies^[15] have found that DCA induces β -catenin signaling increases the expression of cyclin D1 involved in cell cycle progression, degrades tumor suppressor p53, promotes resistance to cell apoptosis, increases cell proliferation and invasion, ultimately leads to the development and further malignant transformation of adenomas^[16]. The study by Liu L *et al* ^[17] provides a new perspective that DCA plays a role in intestinal tumors by regulating the intestinal barrier. By feeding DCA to Apc^{min/+} mice, it was found that the number and size of adenomas in their intestines increased, and the adenoma adenocarcinoma sequence increased. In addition, cytoplasmic tight adhesin-1 (ZO-1) and intestinal cells, such as goblet cells and Paneth cells, were found to be decreased in the intestinal

mucosa of mice treated with DCA. Secretory immunoglobulin A (sIgA) levels were also shown to be significantly reduced. According to the findings, DCA can damage the intestinal mucosa's mechanical and immune defenses, promote cell proliferation, prevent cell apoptosis, and exacerbate the occurrence of intestinal tumors. UDCA is believed to inhibit the occurrence of colonic cancer^[12]. Patients with colonic adenomas who have taken UDCA for a long time have a lower probability of recurrence after resection of colonic adenomas, and the proliferation of colonic epithelium is significantly reduced^[18]. In an experimental mouse colon cancer AOM model, Sharad Khare *et al* ^[19] discovered that DCA greatly promotes tumor formation, but UDCA can inhibit DCA-induced p38 activation and reduce C/EBP β upregulation of Cox-2, hence limiting the carcinogenesis of AOM. In addition, AP-1 and NF- κ B activation caused by DCA can likewise be inhibited by UDCA^[20]. Interventions targeting NF- κ B and AP-1 may play an important role in inhibiting the growth of colonic cancer. ¹¹ The Hippo/YAP pathway plays an important role in the development of cancer. In AOM/DSS induced colonic cancer models, UDCA can be found to reduce YAP expression in a concentration dependent manner, inhibiting tumor growth ^[21]. In this study, the serum DCA content of patients with colonic polyps was lower than that of the control group (colon polyp group: 142.00 (30.90424.25), control group: 234.00 (82.60502.00), $P = 0.011$), while the UDCA content in the colonic polyp group was higher than that in the control group (colon polyp group: 73.70 (23.03221.50), control group: 70.70 (19.00199.00), $P = 0.545$). In other words, it can be considered that in this situation, the DCA content in the intestinal contents of colonic polyp patients increases, while the UDCA content decreases, which is consistent with the above mechanism. However, this study did not actually analyze the BA levels in the feces of colonic polyp patients and healthy control groups, and this part of the study can be added in future studies.

This study went on to conduct grouping analysis based on a comparison of the BA profile detection results between the colonic polyp group and the control group. The results showed that the CA, CDCA, UDCA, and TUDCA contents of the adenomatous polyp group were lower than those of the non adenomatous polyp group. The content

of CA, CDCA, UDCA, GUDCA, TUDCA in the group with polyp diameter ≥ 1 cm was higher than that in the group with polyp diameter < 1 cm. The GCDCA content in the right colon group was significantly higher than that in the left colon group, and the CA content in the left colon group was significantly higher than that in the whole colon group. The CA, GCA, and GCDCA content in the right colon group was significantly higher than that in the whole colon group. The content of CA, CDCA, UDCA, and GUDCA in the group with pedicle polyps was significantly higher than that in the group without pedicle polyps. In the study by Kawano *et al* [10], they monitored the tumor group and discovered that, compared to the subgroup with low DCA levels, the high DCA subgroup had a higher risk of large adenomas (> 3 mm) recurring after four years, and this trend was more pronounced in the left colon. According to Cai Y *et al* [22], right colon tumors had much higher levels of the 12 bile acids than left colon tumors did. In addition, in male patients, the secondary bile acids (DCA, LCA, UDCA) of the right colonic tumor increased compared to the left colonic tumor, but no difference in tumor location was observed in women. Research has shown that the distribution of BA abundance in cancer patients is specific to tumor location, age, and gender, and is related to patient prognosis. From the perspective of pathological characteristics of polyps, this study found that the changes in BA profile are closely related to polyp diameter, polyp site, polyp morphology, pathological type, *etc.* However, the specific role relationship is still unclear, which may be related to the small sample size included in this study. Due to the retrospective nature of this study, additional confounding factors such as inconsistent colonoscopy operators, inconsistent current gastrointestinal symptoms, inconsistent past medical histories of patients, and mismatched colonic polyp group and control group may also have an effect on the research results. However, by taking into account the pathological characteristics of colonic polyps, this study offers new suggestions for the treatment of individuals with colonic cancer.

In summary, the serum BA profile showed significant changes in patients with colonic polyps. The etiology of colon cancers may be intimately associated with secondary bile acid DCA, one of them. At present, the widely recognized view on the

role of serum BA metabolism in the occurrence and development of colon polyps is that BA can induce changes in the colon environment by activating various signaling pathways in the body, thereby promoting the occurrence of colonic polyps and even colonic cancer. Among them, a large number of studies have shown that DCA can induce NF- κ B activation, β -catenin signaling and regulation of intestinal barrier to promote the development of adenomas and the formation of adenocarcinoma. And UDCA can inhibit tumor growth by inhibiting DCA induced NF- κ B activation and inhibiting YAP signaling. However, there is still controversy about whether other components in the BA spectrum can become therapeutic targets for colonic tumors, and further research is needed. This study indicates that controlling the content and composition of serum BA in the absence of intestinal abnormalities, even during the stage of colonic polyps, can to some extent reduce the production of polyps and prevent them from further developing into cancer. In addition, this study provides a new and effective approach for disease screening and postoperative follow-up of colonic polyps from the perspective of characteristic changes in serum BA profile. There are also many shortcomings in this study, and further improvement is needed in future experimental design. Further in-depth research can be conducted by expanding the sample size, collecting fecal samples, and collaborating with other hospitals to conduct multicenter studies, providing a basis for finding effective targets to reduce the production of colonic polyps and the incidence of colonic cancer.

CONCLUSION

This study shows that the serum BA profile of patients with colonic polyps has changed compared to normal individuals. The serum GCA, GCDCA, TCA, and TCDCA levels in the colonic polyp group are significantly higher than those in the control group ($P<0.05$), while the DCA content is lower than that in the control group. Patients with various polyp sizes, locations, morphologies, and pathological types had variable serum BA profile, according to subgroup study of colonic polyps. Therefore, analyzing the

changes in serum BA profile may provide new ideas for finding new targets for the treatment of colonic tumors.

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