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The Clinical Significance of the Expression of the Serum BMP 6 in the Patients with Tumor-related Anemia with Lower Levels of CRP and Hepcidin

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Abstract: *Objective:* To research the expression characteristic and clinical significance of the serum bone morphogenetic protein 6 (BMP6) at the lower expression condition of serum C-reactive protein (CRP) and Hepcidin (Hepc) in patients with tumor-related anemia. *Methods:* 113 hospitalized tumor patients from October 2019 to June 2020 and 20 healthy volunteers (control group) were recruited. We used enzyme-linked immunosorbent assay (ELISA) to determine the levels of BMP6, soluble-hemojuvelin (s-HJV) and Hepc, Electrochemiluminescence method to determine SF level, and particle-enhanced immune transmission turbidity method to determine CRP level, then analyzed their expression characteristics, correlations and clinical significance. *Results:* (1) The levels of BMP6, Hepc and CRP of anemia group were respectively 426.18±202.83mg/mL, 6.58±5.41ug/L and 19.10±18.24mg/L, which were all higher than 334.37±171.32mg/mL, 4.60±2.28ug/L and 3.62±3.46mg/L of non-anemia group (t=2.5804, 2.4750 and 3.4144, P<0.01, P<0.05 and P<0.01, respectively). (2) The level of s-HJV was 0.71±0.28ng/mL, which was obviously lower than 1.07±1.01ng/mL (t=2.6529, P<0.01) in non-anemia group. (3) There were negative correlations between BMP6 and both Hb and s-HJV (respectively r=-0.2807, t=3.0812 and r=-0.2497, t=2.7230, respectively, P both <0.01), but there were no correlations between BMP6 and CRP, Hepc and SF. (4) Hepc was positively correlated with both CRP and SF (r=0.2004, t=2.0660 and r=0.3089, t=3.1045, P<0.05 and P<0.01, respectively). Hepc was negatively correlated with Hb (r=-0.2524, t=2.7525, P<0.01). Hepc had no correlation with s-HJV (P>0.05). (5) The BMP6 in the normal CRP group was 461.86±142.91 mg/mL, which was significantly higher than 364.51± 129.84 mg/mL in elevated CRP group (F=11.35, P<0.01). (6) Hepc, s-HJV and SF in the normal CRP group were 5.17±3.49ug/L, 0.69±0.26ng/mL and 149.89±142.34ng/mL, respectively, which were all significantly lower than 9.02±7.14ug/L, 0.76±0.33ng/mL and 288.36±254.51ng/mL in the elevated CRP group (F=9.56, 4.67 and 2.84, P=0.000, 0.09, 0.046, respectively). *Conclusions:* When tumor patients with anemia is in a low-level condition of CRP and Hepc, the high expression of BMP6 is an important cause for the occurrence and development of anemia.

Keywords: Tumor, Anemia, C-reactive Protein, Bone Morphogenetic Protein 6, Hepcidin

1. Introduction

Existing studies [1-3] have shown that C-reactive protein (CRP) is one of the important inflammatory factors in tumor patients, which can cause high expression of Hepcidin (Hepc) in tumor patients, then leads to the body's deavailability of iron and cause anemia. At present, the interleukin-6 (IL-6)—CRP—Hepc pathway is one of the significant mechanisms for the development of anemia of chronic diseases (ACD), including tumor-related anemia. And its relationship with tumor-related anemia has been basically clear [4, 5]. It has been reported that Bone Morphogenetic Protein 6 (BMP6) is also associated with ACD [6], but its relationship with CRP and Hepc is not well studied. Therefore,

the serum BMP6, CRP, Hepc, soluble-hemojuvelin (s-HJV), serum ferritin (SF) and hemoglobin (Hb) levels in 113 tumor patients were determined in order to explore the expression characteristics of BMP6, CRP and Hepc in tumor patients, their correlation with the development of anemia and their clinical significance.

2. Materials and Methods

2.1. Research Subjects

A total of 113 tumor patients were admitted to our study from October 2019 to June 2020 in our hospital. Among them, there were 52 males and 61 females, aged from 23 to 80 years old, with an average age of 61.4 years. The tumor types included 44 cases of gastric cancer, 35 cases of breast cancer, 22 cases of bowel cancer, 7 cases of lung cancer and 5 cases of lymphoma. And we selected 20 healthy volunteers in the same period as a control group (a comprehensive examination was carried out on volunteers after recruitment to exclude various diseases, especially those affecting iron metabolism, such as chronic kidney diseases, liver injury and other diseases [7-10]). These 113 patients were divided into two groups according to whether there was anemia [anemia group (60 cases) and non-anemia group (53 cases)]. And further, 60 anemia patients were divided into two subgroups according to CRP expression level [normal CRP group (38 cases) and elevated CRP group (22 cases)] for comparative analysis.

2.2. Instruments and Reagents

CRP reagents were purchased from DiaSys Diagnostic System GmbH company, and the detecting instrument was Siemens fully automated biochemical instrument. BMP6, Hepc and s-HJV reagents were supplied by Suzhou Yueya Biotechnology Co Ltd., and the detecting instrument was the Finnish labsystems Multiskan MS (Model 352). The detection reagent of SF was provided by Roche, and the detecting instrument was Roche COBAS601 electrochemiluminescence meter. And the Coulter-Beckman Pentaxic Blood Routine Tester was used to determine Hb.

2.3. Methods

(1) Avidin-Biotin Complex-enzyme linked immunosorbent assay (ABC-ELISA) method was used to determine BMP6, s-HJV and Hepc. (2) Electrochemiluminescence method was used to determine SF. (3) Particle-enhanced immune transmission turbidity method was used to determine CRP. (4) Conventional method was used to determine Hb.

2.4. Statistical Analysis

Through software SPSS 23.0, chi-square test, t-test, and one-way ANOVA (F-test) were used to compare mean differences between multiple groups, q-test was used for the two-by-two comparison between multiple means, and the Pearson correlation analysis was used for correlation analysis. The results were statistically significant when $P<0.05$.

3. Results

3.1. The Determination Results of Serum BMP6, CRP, Hepc, s-HJV and SF in 113 Patients

The determination results of BMP6, CRP, Hepc, s-HJV, SF and Hb in 113 patients are shown in Table 1. The BMP6, CRP and Hepc levels of the anemia group were all significantly higher than those in the non-anemia group and the s-HJV level was lower than that in the non-anemia group, of which were all statistically significant.

Among the 60 patients of anemia group, 22 cases had an increase in CRP level and 38 cases with normal CRP level, while in the 53 cases without anemia 4 cases had an increase in CRP level and 49 cases with normal CRP level. The number of patients with elevated CRP level in anemia group was significantly more than that in non-anemia group ($\chi^2=7.61$, $P<0.01$).

Table 1. *Determination and comparison results of BMP6 and other indicators in 113 patients (x±s, t-test).*

Determination items	Anemia group (n=60)	Non-anemia group (n=53)	t value
Hb (g/L)	97.82±15.42	126.58±9.42	11.7748**
BMP6 (mg/mL)	426.18±202.83	334.37±171.32	2.5804**
CRP (mg/L)	19.10±18.24	3.62±3.46	3.4144**
Hepc (ug/L)	6.58±5.41	4.60±2.28	2.4750*
s-HJV (ng/mL)	0.71±0.28	1.07±1.01	2.6529**
SF (ng/mL)	200.67±164.79	162.89±160.12	0.8931

Note: *$P<0.05$, **$P<0.01$, the rest $P>0.05$

3.2. Analysis Results

3.2.1. Correlations Between BMP6 and CRP, Hepc, Hb, SF and s-HJV

The analysis results of correlations between BMP and CRP and other indicators in 113 patients showed that BMP6 was negatively correlated with Hb and s-HJV ($r=-0.2807$, $t=3.0812$ and $r=-0.2497$, $t=2.7230$, respectively, $P<0.01$). BMP6 had no correlations with CRP, Hepc, and SF ($r=-0.0147$, -0.0612, and 0.0069, respectively, P all >0.05).

3.2.2. Correlations Between Hepc and CRP, Hb, SF, s-HJV

Correlation analysis between Hepc and CRP and other indicators showed that Hepc was positively correlated with CRP and SF ($r=0.2004$, $t=2.0660$ and $r=0.3089$, $t=3.1045$, $P<0.05$ and $P<0.01$, respectively). Hepc was negatively correlated with Hb ($r=-0.2524$, $t=2.7525$, $P<0.01$). Hepc had no correlation with s-HJV ($r=0.1396$, $P>0.05$).

3.3. 60 Anemia Patients' Determination Results and Comparisons of BMP6 and Other Indicators in Groups Grouped According to the CRP Level

According to the CRP level, we divided 60 patients with anemia into normal CRP group (38 cases) and elevated CRP group (22 cases), and compared them with the control group (20 cases) (F test). The specific results are shown in Table 2. Because only 4 of the 53 patients without anemia had elevated CRP, no analysis between normal and elevated CRP groups

was performed in patients without anemia.

Table 2. *60 anemia patients' determination results and comparison of BMP6 and other indicators in groups grouped according to the CRP level (x±s, F/P and q/P).*

Items	normal CRP group	elevated CRP group	control group	F/p	control/normal CRP group	control/elevated CRP group	normal/elevated CRP group
n	38	22	20		(q/P)	(q/P)	(q/P)
Hb (g/L)	98.34±15.94	96.91±14.39	134.81±10.90	77.56/0.000	12.80/0.000	11.88/0.000	0.49/0.670
BMP6 (mg/mL)	461.86±142.91	364.51±129.84	365.76±145.61	11.35/0.000	3.55/0.000	0.04/0.903	3.71/0.044
Hepc (ug/L)	5.17±3.49	9.02±7.14	3.17±1.91	9.56/0.000	2.27/0.069	5.91/0.000	4.48/0.011
s-HJV (ng/mL)	0.69±0.26	0.76±0.33	1.01±0.38	4.67/0.009	4.57/0.000	3.13/0.039	1.00/0.865
SF (ng/mL)	149.89±142.34	288.36±254.51	178.85±103.32	2.84/0.046	0.64/0.712	2.18/0.059	3.17/0.041
CRP (mg/L)	2.58±2.26	47.66±39.54	1.22±0.22	35.73/0.000	0.33/0.465	9.99/0.000	11.19/0.000

4. Discussion

In this study, BMP6, Hepc and CRP were significantly higher in anemia group than those in non-anemia group, while s-HJV was significantly lower in anemia group, indicating that BMP6, Hepc and CRP, as well as s-HJV, were all related to the development of anemia in tumor patients. Our further analysis showed that Hepc was negatively correlated with Hb and positively correlated with both CRP and SF. This result is consistent with our previous research results [1, 11] and foreign results [4-6, 12, 13], demonstrating that the inflammatory response in tumor patients leads to high expression of Hepc, which in turn causes the body's deavailability of iron and eventually leads to anemia.

BMP6 was negatively associated with Hb, and had no correlation with Hepc, CRP and SF, which is consistent with our past results [6], suggesting that although BMP6 was also related to the occurrence of anemia, anemia was not caused by the CRP-Hepc pathway, but by other different mechanisms [14-16]. According to the CRP level, 60 anemia patients were divided into the normal CRP group and the elevated CRP group, and further analysis found that the Hb levels of the two groups were basically the same, but the BMP6 levels of the normal CRP group were significantly higher than that in the elevated CRP group, while Hepc and SF were significantly lower than those in the elevated CRP group, which further suggested that BMP6 was an important cause for the patients to develop anemia in the low-level condition of CRP or Hepc (that is, in the condition without obvious inflammatory response).

Meanwhile, our study also found that BMP6 was negatively correlated with s-HJV, which was consistent with previous researches [17], but whether the negative regulatory relationship on BMP6 was lost due to low expression of s-HJV, or whether the high expression of BMP6 inhibited the expression of s-HJV, it cannot be fully explained [18, 19]. s-HJV had no directly correlation with Hb, in the anemia group, it was significantly lower than that in the non-anemia group [20], and in the normal CRP group of anemia patients s-HJV was also lower than that in the elevated CRP group and even much lower in the control group, suggesting that it may cause a negative compensatory response after anemia caused by high expression of BMP6. The exact relationship needs to be further studied.

Of course, some patients may have both the CRP-Hepc pathway and the BMP6 pathway, which was reflected in the fact that the Hepc of patients in the normal CRP group in this study was slightly higher than that in the control group.

5. Conclusion

In conclusion, in the occurrence and development of anemia in tumor patients, Hepc and BMP6 cause anemia through two different mechanisms. This is of great significance for the treatment of anemia in tumor patients, such as Hepc-related anemia can be effectively treated by applying Hepc-specific monoclonal antibodies or low molecular weight heparin, while drugs such as monoclonal antibodies for BMP6 are effective for anemia caused by the BMP6 pathway, so it is worth further in-depth research.

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