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# Clinical and Experimental Study of Low Molecular Weight Heparin in Patients with Chronic Anemia

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## Abstract

**Objective:** To preliminary study the significance of low molecular weight heparin (LMWH) in the treatment patients of with anemia of chronic diseases (ACD), and the changes in the serum levels of BMP6, hepcidin and IL-6. To preliminary study the significance of low molecular weight heparin (LMWH) in the treatment the patients with anemia of chronic diseases (ACD), and the changes in the serum levels of BMP6, hepcidin and IL-6. **Methods:** Used LMWH (4000 u/day, 7 - 15 days) to therapy 61 patients with ACD, and ELISA method was used to determine Hepcidin and BMP6 before and after treatment, and the determination of IL-6 by Electro-chemi-luminescence, and to analyze its clinical significance. **Results:** 1) In all 61 cases, the levels of Hepcidin in post-therapy were 0.82 ± 0.24 mg/L, which were lower than 1.05 ± 3.83 mg/L in pre-therapy (t = 2.5726, $P < 0.05$). The levels of IL-6 in post-therapy were 24.88 ± 12.58 mg/L, which were lower than 38.22 ± 31.23 mg/L in pre-therapy (t = 2.9650, $P < 0.05$), but there were no statistically significant both Hb and BMP6 between in pre-therapy and post-therapy (all $P > 0.05$). However, The levels of Hb in post-therapy were higher than in pre-therapy (t = 1.9832, $P < 0.05$). 2) The Hb level in the tumor anemia group after treatment was 91.18 ± 15.91 g/L, which was higher than that before treatment (85.45 ± 18.33 g/L), the difference was statistically significant (t = 1.9711, $P < 0.05$). 3) The levels of hepcidin and IL-6 in the tumor anemia group after treatment were 0.73 ± 0.45 mg/L and 30.33 ± 28.39 mg/ml, which were lower than those before treatment (1.09 ± 0.41 mg/L and 50.76 ± 42.10 mg/ml), respectively, the difference was statistically significant (t = 3.3941, $P < 0.01$ and t = 2.3597, $P < 0.05$). 4) There was no significant difference in all indexes in tumor anemia free group (all $P > 0.05$). 5) Although Hb level increased

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slightly in the non-tumor anemia group, there was no statistical significance ($P > 0.05$), and there was no statistical difference in other indexes (all $P > 0.05$). 6) After treatment, the level of Hb was negatively correlated with Hepcidin and IL-6 (respectively r = −0.2809, t = 2.2490, $P < 0.05$ and r = −0.2781, t = 2.2266, $P < 0.05$). Hepcidin was positively related to IL-6 (r = −0.2941, t = 2.3622, $P < 0.05$). There was no correlation between BMP6 and Hb, Hepcidin and IL-6 levels. **Conclusion:** LMWH could up-regulate the levels of Hb, and better for the degree of anemia in patients with ACD. The possible mechanism is to reduce the level of Hepcidin and IL-6.

## Keywords

Anemia of Chronic Disease, BMP6, Hepcidin, IL-6, Hemoglobin, Low Molecular Weight Heparin, Therapy

## 1. Introduction

At present, it has been confirmed that the inflammatory response of patients with chronic anemia (anemia of chronic disease, ACD) leads to the high expression of interleukin-6 (interleukin-6, IL-6) and the high expression of ferritin (also known as a liver antimicrobial polypeptide, Hepcidin, referred to as Hep), and finally leads to anemia. However, the treatment of ACD is very difficult. Recently, it has been reported that heparin can improve anemia in ACD. The mechanism may be that heparin down-regulates the expression of Hep through the bone morphogenic protein 6 (bone morphogenetic protein 6, BMP6) pathway. The observation that BMPs are heparin-binding molecules and that heparin modifies the osteogenic activity of BMP2/4 stimulated (Poli *et al.*, 2011) to verify the effect of heparin on hepcidin expression. It was shown that commercial heparins are potent hepcidin inhibitors *in vitro* in HepG2 cells and *in vivo* in healthy mice and that act by inhibiting the BMP6/SMAD signaling. Heparins are well-characterized molecules with some 70 years of clinical experience, and appealing drugs for the treatment of anemia. However, there are few studies on this aspect in our country. Based on early clinical observation of low molecular weight heparin (low molecular weight Heparin, LMWH) in the treatment of tumor-associated anemia, we further observed the changes of hemoglobin (Hb) level, BMP6, IL-6, and Hepcidin in patients with ACD after LMWH treatment. To analyze the clinical significance and possible mechanism of LMWH in the treatment of ACD.

## 2. Materials and Methods

### 2.1. Research Object

There were 61 inpatients in our hospital from January 2016 to June 2018, including 32 males and 29 females, aged 33 - 90 years, with an average age of 61.7 years. All patients were treated with LMWH because of thrombosis or high

plasma D-dimer with thrombotic tendency. The types of diseases were as follows: 1) 43 cases of malignant tumors (including 11 cases of lung cancer, 8 cases of gastric cancer, 5 cases of colorectal cancer, 5 cases of ovarian cancer, 3 cases of lymphoma, 3 cases of prostate cancer, 2 cases of breast cancer, 2 cases of liver cancer, 2 cases of pancreatic cancer and 2 cases of cervical cancer). 2) there were 18 cases of non-tumor diseases (including 10 cases of acute myocardial infarction and acute coronary syndrome, 6 cases of varicose veins with thrombosis of lower extremities, 1 case of severe pneumonia, and 1 case of pulmonary embolism). 3) the cases of malnutrition and chronic hepatorenal diseases with obvious influence on iron metabolism have been excluded, and the cases of less than 7 days of heparin treatment have also been removed.

### 2.2. Reagents and Instruments

1) Reagents are purchased from DiaSys Diagnostic System Gmb, including human hepc ELISA Kit (96T) and Elecsys and codebase analyzers (IL-6. 100T) and Human Bone Morphogenetic Protein 6ELISA kit (96T), etc. 2) The detection instrument used is the enzyme labeling instrument of American BioTex Company, the electro-chemi-luminescence instrument of ELx800; Roche Company, and the model COBAS601.

### 2.3. Research Methods

1) Grouping method: the patients were divided into tumor group and non-tumor group, and each group was divided into anemia group and non-anemic group according to the level of Hb. There were 43 cases in the tumor group, including 33 cases of anemia and 10 cases of no anemia, 18 cases of the non-tumor group, 9 cases of anemia, and 9 cases of non-anemia. The diagnostic criteria of anemia were Hb < 120.0 g/L in males and <110.0 g/L in females. 2) low molecular weight heparin therapy: farming (Pfizer) or enoxaparin (Hangzhou Jiuyuan Gene Biology Co., Ltd.) were used for 4000 u/days, subcutaneously injected for 7 - 15 days for at least 7 days. Blood samples were taken to detect Hb on the day before treatment and the day after treatment. 3) Test method: the blood samples of fasting patients were taken in the early morning and stored in different test tubes, stored in a refrigerator at −80˚C, and monitored centrally at the same time. Hepcidin and BMP6 were detected by the ELISA method and IL-6, Hb was detected by the electro-chemi-luminescence method.

### 2.4. Statistical Analysis

SPSS19.0 software package was used for statistical analysis. T-test and correlation analysis were used respectively. *P*-value < 0.05 was considered to be statistically significant.

## 3. Results

The results of Hb level and various detection indexes of all 61 patients before

and after LMWH treatment are shown in Table 1. Hb and BMP6, Hepcidin, and IL-6 were measured before and after LMWH treatment. The level of Hepcidin, IL-6 after treatment was lower than that before treatment, and the difference was statistically significant ($P < 0.05$), but there was no significant difference between Hb and BMP6 ($P > 0.05$).

The level of Hb and the detection results of various indexes in tumor and non-tumor patients before and after LMWH treatment are shown in Table 2. Considering the difference between the nature of the tumor and non-tumor disease, the two were compared and analyzed respectively. The Hb level in the tumor anemia group after treatment was 91.18 ± 15.91 g/L, which was higher than that before treatment (85.45 ± 18.33 g/L), the difference was statistically significant (t = 1.9711, $P < 0.05$). The levels of hepcidin and IL-6 in the tumor anemia group after treatment were 0.73 ± 0.45 mg/L and 30.33 ± 28.39 mg/ml, which were lower than those before treatment (1.09 ± 0.41 mg/L and 50.76 ± 42.10 mg/ml), respectively, the difference was statistically significant (t = 3.3941, $P < 0.01$ and t = 2.3597, $P < 0.05$). There was no significant difference in all indexes in the tumor anemia-free group (all $P > 0.05$). Although the Hb level increased slightly in the non-tumor anemia group, there was no statistical significance ($P > 0.05$), and there was no statistical difference in other indexes (all $P > 0.05$).

The correlation analysis is shown in Table 3. The correlation between Hb level and BMP6, IL-6, and Hepcidin in 61 patients after treatment was analyzed. The results showed that 1) there was a negative correlation between Hb level and IL-6 and Hepcidin, but no correlation with BMP6. 2) IL-6 was positively correlated with Hepcidin, but not with BMP6.

**Table 1.** Determination results of various indexes in all 61 patients before and after treatment ( $\overline{\chi} \pm S$ ).

| Item | Subgroup | n | Before treatment | After treatment | t | *P* |
|---|---|---|---|---|---|---|
| Hb (g/L) | | 61 | 96.62 ± 25.24 | 98.67 ± 21.59 | 0.8482 | >0.05 |
| | anemia | 42 | 84.57 ± 27.13 | 89.74 ± 25.26 | 1.9832 | <0.05 |
| | Non-anaemia | 19 | 123.26 ± 31.12 | 118.37 ± 21.19 | 1.5866 | >0.05 |
| BMP6 (mg/ml) | | 61 | 162.49 ± 86.35 | 158.61 ± 81.01 | 0.3424 | >0.05 |
| | anemia | 42 | 173.70 ± 82.33 | 168.66 ± 83.08 | 0.9647 | >0.05 |
| | Non-anaemia | 19 | 137.72 ± 66.23 | 136.39 ± 71.25 | 0.0158 | >0.05 |
| Hepcidin (mg/L) | | 61 | 1.02 ± 3.83 | 0.82 ± 0.24 | 2.5726 | <0.05 |
| | anemia | 42 | 1.05 ± 3.96 | 0.74 ± 0.19 | 2.9865 | <0.01 |
| | Non-anaemia | 19 | 0.94 ± 3.22 | 0.96 ± 0.31 | 0.3514 | >0.05 |
| IL-6 (mg/ml) | | 61 | 38.22 ± 31.23 | 24.88 ± 12.58 | 2.9650 | <0.01 |
| | anemia | 42 | 43.96 ± 33.42 | 26.60 ± 23.36 | 2.6715 | <0.01 |
| | Non-anaemia | 19 | 33.42 ± 23.17 | 17.88 ± 13.18 | 3.1688 | <0.01 |

**Table 2.** Results of determination of various indexes in tumor and non-tumor patients before and after treatment.

| Item | Tumor group (n = 43) | | | | | | Non-tumor group (n = 18) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Anemia group (n = 33) | | | Non-anemia group (n = 10) | | | Anemia group (n = 9) | | | Non-anemia group (n = 9) | | |
| | Before treatment | After treatment | t | Before treatment | After treatment | t | Before treatment | After treatment | t | Before treatment | After treatment | t |
| Hb (g/L) | 85.45 ± 18.33 | 91.18 ± 15.91 | 1.9711* | 122.80 ± 7.08 | 118.40 ± 11.47 | 1.0320 | 81.33 ± 39.04 | 84.44 ± 37.27 | 0.1729 | 123.78 ± 47.34 | 118.33 ± 47.17 | 0.2446 |
| BMP6 (mg/ml) | 172.60 ± 99.80 | 167.76 ± 94.00 | 0.1838 | 155.74 ± 47.10 | 151.42 ± 37.48 | 0.2270 | 177.74 ± 77.69 | 171.96 ± 88.19 | 0.1312 | 117.69 ± 59.17 | 119.69 ± 59.17 | 0.0480 |
| Hepcidin (mg/L) | 1.09 ± 0.41 | 0.73 ± 0.45 | 3.3941# | 1.07 ± 0.32 | 1.07 ± 0.33 | 0.0010 | 0.89 ± 0.52 | 0.80 ± 0.51 | 0.3721 | 79 ± 0.41 | 0.84 ± 0.48 | 0.2240 |
| IL-6 (mg/ml) | 50.76 ± 42.10 | 30.33 ± 28.39 | 2.3597* | 37.57 ± 45.37 | 25.29 ± 18.92 | 1.4334 | 19.01 ± 15.72 | 19.72 ± 812.4 | 0.0809 | 14.41 ± 12.60 | 39.64 ± 7.45 | 1.6015 |

Note: *$P < 0.05$, #$P < 0.01$, others are $P > 0.05$.

**Table 3.** Correlation analysis results.

| Comparison group | r | *P* | Comparison group | r | *P* |
|---|---|---|---|---|---|
| Hb/BMP6 | −0.0026 | >0.05 | Hepcidin/IL-6 | 0.2941* | <0.05 |
| Hb/Hepcidin | −0.2809* | <0.05 | Hepcidin/BMP6 | 0.0447 | >0.05 |
| Hb/IL-6 | −0.2781* | <0.05 | IL-6/BMP6 | 0.0770 | >0.05 |

Note: *$P < 0.05$, others are $P > 0.05$.

## 4. Discussion

Existing studies have shown that malignant tumor is a chronic inflammatory disease, and tumor-associated anemia is a kind of ACD. The high expression of IL-6 and Hepcidin in serum of these patients is the main cause of anemia [1] [2] [3] [4]. However, most of the tumor patients with anemia are actually in the advanced stage and lack effective treatment. Therefore, it is difficult to control the inflammatory state of ACD by treating the primary disease of a tumor. Therefore, the application of anti-IL-6 or anti-Hep in the treatment of ACD is a subject worthy of further study, and it is likely to be the hope for the treatment of tumor ACD [5] [6] [7]. Recently, some foreign scholars (MUARA *et al.*) have observed the changes of Hb in the process of using heparin in some patients with pneumonia and heart disease and then found that it is related to the changes of Hepcidin level and Hb level [8].

This study showed that the level of Hb in the tumor anemia group after treatment was 91.18 ± 15.91 g/L, which was higher than that before treatment (85.45 ± 18.83 g/L) (t = 1.971, $P < 0.05$). At the same time, the levels of IL-6 and Hepcidin were significantly lower than those before treatment, and the difference was statistically significant (t = 2.3597, $P < 0.05$ and t = 3.3941, $P < 0.01$). This is consistent with the results of our recently reported study [9]. Further research and analysis showed that the levels of IL-6 and Hepcidin were negatively correlated with Hb levels, while the levels of IL-6 and Hepcidin were positively

correlated. This result is consistent with our previous research results; it is also consistent with the results of MAURA *et al.*, and MAURA *et al.* found that Hepcidin decreased significantly in all subjects after the administration of heparin (but they did not detect IL-6), indicating that heparin can increase the level of Hb in tumor patients and improve the state of anemia.

At the same time, this study also observed the changes of various indexes in 18 non-tumor patients after using LMWH, but except for the slight increase of Hb level in the anemia group, the differences of all other indexes were not statistically significant, which was different from the results of MUARA *et al.* The reason for the analysis may be related to different types of diseases, including 3 cases of pneumonia, 1 case of chronic heart failure and 1 case of chronic heart failure complicated with pneumonia, while we are mainly acute diseases of acute myocardial infarction, acute coronary syndrome and vascular diseases of lower extremities. At the same time, the further study found that the serum IL-6 level of the 18 non-tumor patients was generally lower than that of tumor ACD patients, suggesting that the inflammatory state of the body is not serious.

Foreign studies have shown that heparin can improve anemia mainly through BMP6 down-regulating the level of Hepcidin [10] [11]. The mechanism may be related to the inhibition of BMP6-mediated Hepcidin expression and the inhibition of the IL-6 pathway to down-regulate the level of Hepcidin. However, we found that although the BMP6 level of tumor ACD patients seemed to be higher than that of non-tumor patients, there was no significant difference in BMP6 levels between anemic and non-anemic patients before and after treatment (all $P > 0.05$). There was no correlation between BMP6 and the levels of Hepcidin and Hb (all $P > 0.05$), suggesting that the mechanism of anemia may not be the same as that of other ACD in patients with tumor ACD and patients with acute cardiovascular disease without chronic inflammation. We will further study the exact significance of the different expressions of BMP6 in tumor ACD patients and non-tumor patients.

In addition, this study found that the improvement of the Hb level of patients by LMWH was not as obvious as that reported in the literature. The possible reasons were related to different heparin preparations. LMWH was used in this study. It is reported that unfractionated heparin is used in literature. This study shows that unfractionated heparin has a strong dose-dependent effect. Unfractionated heparin is about 10 times more potent than LM-WH, that is, the ability to increase the level of Hb is in the following order: unfractionated heparin (molecular weight 12 - 15 kDa) > enoxaparin (4.5 kDa) > pentasaccharide sulfa heparin (1.7 kDa). We also need to focus on adverse reactions, because our patient may still have had an underlying coagulopathy. For example, unfractionated heparin may have placed the patient at an increased risk for an epidural hematoma. This is especially prudent in the setting of TID-dosed subcutaneous heparin [12].

In a word, LMWH can improve the anemia of tumor ACD patients to some extent by down-regulating the levels of IL-6 and Hepcidin, which is worthy of

further study.

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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