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Hematological Findings in Cobalamin Deficiency with Special Reference to Hemolysis and Hyperhomocysteinemia

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Abstract
Introduction. *Co-existent hemolysis in patients with vitamin B12 deficiency is a well-recognized phenomenon. Hemolysis in megaloblastic anemia was traditionally thought to be due to intramedullary destruction of RBCs (ineffective erythropoiesis). However, the degree of hemolysis was higher in megaloblastic anemia and significantly correlated with hyperhomocysteinemia. In vitro study revealed that Homocysteine increased the risk of hemolysis in Vitamin B12 deficiency patients while its mechanisms are still not entirely understood and has not been demonstrated in clinical setting.*
Material and methods:*30 patients of Megaloblastic anemia with hemolysis were taken as cases and and 30 cases of megaloblastic anemia without hemolysis were taken as control. Complete hemogram, schistocyte count (expressed in percentage) along with biochemical investigations (vitamin B12, folic acid, serum LDH, bilirubin, Homocysteine) were done.*
Result: *Hemoglobin (Hb) values(gm/dl) in Megaloblastic anemia with hemolysis and without hemolysis were 5.62 ± 1.4 and 6.14 ± 2. Though mean total leucocyte count and total platelet count were within normal range in both these groups but some of the patients presented with leucopenia and thrombocytopenia. Reticulocyte count was increased i.e. 3.83 ± 1.5 in hemolysis group compared to control group where mean reticulocyte count was 1.06 ± 0.47. Schistocytes count is very much increased in hemolysis group. i.e 7.08 ± 3.19 and it was 1 ± 0.6 in control group. Homocysteine level was more increased in cobalamin deficiency with hemolysis i.e. 37.28 ± 15 as compared to control group where Homocysteine level was 31.15 ± 19. Indirect bilirubin was also increased in hemolytic cases. LDH level was slightly increase in cases where as it is normal in control groups.*
Conclusion:*Cobalamine deficiency causes anemia,leucopenia,thrombocytopenia, pancytopenia as well as hemolytic blood picture. Our study confirms that there is well-known link between vitamin B12 and plasma homocysteine level. Both features of intramedullary destruction and extravascular hemolysis were evident and both hemolysis and pancytopenia as well as hyperhomocysteinemia were corrected by cobalamin treatments. So we hypothesize that high homocysteine level may be an important contributor leading to further hemolysis which is often seen in cobalamin deficiency.*
Keywords: *Megaloblastic Anemia,hemolysis, Hyperhomocystinemia.*

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I. Introduction

Cobalamin (Vitamin B12) deficiency is seen frequently in elderly patients and young children but many a times it is unrecognized or not investigated because the clinical manifestations are subtle. However serious clinical and hematological manifestations need investigations of all patients of vitamin B12 deficiency. Previously cobalamin deficiency was best diagnosed by presence of macro-ovalocytes, hyper segmented neutrophils in peripheral blood smear and progressive increase in mean corpuscular volume. But over last three decades the introduction of automated analysers for measuring serum levels of cobalamin and homocysteine made the diagnosis of cobalamin deficiency easier.

Hematological consequences of cobalamin deficiency can be severe. Approximately 10% of patients had life threatening condition like symptomatic pancytopenia, hemolytic anemia and pseudo thrombotic microangiopathy. (1, 2).Hemolysis in patients with cobalamin deficiency is due to intramedullary destruction but various in vitro studies revealed that increased hemolysis occurs due to hyperhomocysteinemia in vitamin B12 deficiency. In our case series of 30 cases of cobalamin deficiency with features of hemolysis associated with hyperhomocysteinemia. After vitamin B12 treatment, there is normalization of Homocysteine and

correction of hemolysis was seen. So we hypothesize that increased homocysteine level is an important contribution leading to further hemolysis in patients with cobalamin deficiency.

II. Material & Methods

The study was conducted in department of pathology KIMS, KIIT university, Bhubaneswar, Odisha from September 2016 to august 2018 over a period of 2 yrs. The study group was divided into two groups,i.e Megaloblastic anemia patients without hemolytic blood picture and megaloblastic anemia with hemolytic blood picture. Any other conditions showing hemolytic blood picture were excluded from the study. Informed consent taken from each patient. Detailed clinical history from each patient was obtained and recorded. A presumptive diagnosis was made and subjected for different hematological investigations.

Under aseptic precuations 3 ml of fasting venous blood samples was collected with EDTA as anticoagulant. The samples were analysed in automated counter(Beckman coulter LH 750). Hemoglobin, hematocrit, MCV, MCH, MCHC, RDW, WBC count, Platelet count were determined. From the peripheral blood film examination,red cell morphology alteration(macrocytosis, anisopoikilocytosis, schistocytes, polychromasia, nucleated RBC), WBC morphology alteration and platelet morphology were noted. Schistocyte count was done according to ICSH guidelines and noted .Supravital stain was done and reticulocyte was counted.Serum vitamin B12, folic acid and homocysteine level were measured in semiautomatic analyser by ECLIA method. Serum LDH, bilirubin (total and indirect), serum iron profile and HPLC were done..

III. Results And Discussion

30 cases of cobalamin deficiency with hemolytic blood picture were included in our study and 30 consecutive cases of cobalamin deficiency without hemolytic blood picture were taken as control. Features of hemolysis was considered in terms of either increase LDH, increase indirect bilirubin or presence of schistocytes, normoblasts & polychromatic cells in peripheral blood smear.

Detail hematological and biochemical parameters of the cases were seen and compared with the control groups. Hemoglobin (Hb) values in Megaloblastic anemia with hemolysis were 5.62 ± 1.4 and those in control group were 6.14 ± 2. Though mean total leucocyte count and total platelet count were within normal range in both these groups but some of the patients presented with leucopenia and thrombocytopenia. Reticulocyte count was increased i.e. 3.83 ± 1.5 in hemolysis group compared to control group where mean reticulocyte count was 1.06 ± 0.47. Schistocytes count is very much increased in hemolysis group. i.e 7.08 ± 3.19 and it was 1 ± 0.6 in control group.(Table-1)

Table 1.distribution of hematological parameters among clinical control and cases

Parameters	**Clinical control (N = 30) (Mean ± SD)**	**cases (N = 30) (Mean ± SD)**
Hemoglobin	6.1 ± 2.04*	5.6 ± 1.40*
TLC	5034.5± 2214.81	4052±1442.26
TPC	99780 ± 64874	123410± 60821.36
Reticulocyte Count	1.06 ± 0.47	3.83 ± 1.52
schistocytes	1 ± 0.66	7.08 ± 0.66

Cobalamin level was decreased in both the groups (84.4 ± 40.5 in cases and 92.5 ± 79.6 in control group). Homocysteine level was more increased in cobalamin deficiency with hemolysis i.e. 37.28 ± 15 as compared to control group where Homocysteine level was 31.15 ± 19. Indirect bilirubin was also increased in hemolytic cases. LDH level was slightly increase in cases where as it is normal in control groups.(Table-2)

Table 2.distribution of biochemical parameters among clinical control and cases

Parameters	**Clinical control (N = 30) (Mean ± SD)**	**cases (N = 30) (Mean ± SD)**
LDH	236.13 ± 110.10	342.40 ± 127.81
Total Bilirubin	1.15 ± 0.36	1.13 ± 0.57
Vitamin B12	92.49 ± 79.67	84.46 ± 40.54
Folic Acid	5.04 ± 5.70	4.50 ± 4.11
Homocysteine	31.15 ± 19.10	37.28 ± 15.06

This study done from a single institution with consecutive patients with well documented cobalamin deficiency. All the patients in our study fulfilled the criteria of cobalamin deficiency (i.e. serum vitamin B12 levels <200pg/ml). This study shows that almost all patients with cobalamin deficiency have some degree of hematological manifestation in the form of anemia,leucopenia thrombocytopenia. In our study reticulocyte

count was increased i.e. 3.83 ± 1.5 in hemolysis group compared to control group where mean reticulocyte count was 1.06 ± 0.47. Indirect bilirubin and LDH level were also increased in hemolytic cases, where as it is normal in control groups. It indicates that intramedullary hemolysis is occuring in hemolytic groups.

Schistocytes count is very much increased in hemolysis group. i.e 7.08 ± 3.19 and it was 1 ± 0.6 in control group. Homocysteine level was also more increased in cobalamin deficiency with hemolysis i.e. 37.28 ± 15 as compared to control group where Homocysteine level was 31.15 ± 19.So it clearly indicates that increase homocysteine level might causing intravascular hemolysis and inturn producing more scistocytes in the peripheral blood.Secondly normalisation of schistocytes count occures after giving cobalamine treatment.

Though the mechanism is not clearly understood but hemolysis in patients with cobalamin deficiency is a well-recognized phenomenon. It is assumed that hemolysis in Vitamin B12 diet is due to intramedullary hemolysis

In our study both features of intramedullary destruction and extravascular hemolysis were evident and both hemolysis and pancytopenia as well as hyperhomocysteinemia were corrected by cobalamin treatments. So we hypothesize that high homocysteine level may be an important contributor leading to further hemolysis which is often seen in cobalamin deficiency.

3.1

Homocysteine has been proposed as a hemolytic toxin. (3,4) However, the exact mechanism of hemolytic effects of homocysteine is not clear. (3,4) It is assumed that high homocysteine level leads to endothelial damage with ensuring microangiopathy causing hemolysis. Evidences suggest that hyperhomocysteinemia is associated with thrombosis (5,6,) and endothelial damage or dysfunction. (6,7)The prooxidant effects of homocysteine were presumed as the likely of endothelial damage. Elevated homocysteine levels have been described in literature as a possible etiology for both intravascular and intramedullary hemolysis with the role of homocysteine in increasing the risk of hemolysis in vitamin B12 deficiency being demonstrated in vitro. (8)

IV. Conclusion

Hemolysis in cobalamin deficiency is though a rare presentation but requires high clinical suspicion for early diagnosis and treatment. So in any case of hemolytic blood picture focus should be given for presence of macroovalocytes and hypersegmented neutrophils to rule out more common Megaloblastic anemia.

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