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Autoimmune Hemolytic Anemia as a Rare Adverse Effect of Hydroxyurea in Sickle Cell Anemia -A Case Report

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ABSTRACT: Hydroxyurea is a medication that is commonly used in the treatment of various hematological disorders, including sickle cell anemia .Hydroxyurea remains the main and well tolerated drug used for sickle cell anemia. The mechanism of action of Hydroxyurea is through impairment of DNA synthesis by blocking the ribonucleoside diphosphate reductase need for synthesis of RNA thus, preventing conversion of ribonucleotides to deoxyribonucleotides. Henceforth as a result there may be neutropenia, followed by anemia with megaloblastosis and thrombocytopenia. This case report indicates that hydroxyurea can propably cause hemolytic anemia however the exact mechanisms is not known. Further toxicological studies should be done to prove it as an adverse effect.

Keywords : Hydroxyurea, Sickle cell Disease , Hemolytic Anemia , Coombs Test

I. INTRODUCTION :

Sickle cell anemia is a prevalent and severe autosomal inherited blood condition that affects populations worldwide. Sickle cell anemia is the result of having two copies of the beta-S (βS) allele, which is found on chromosome 11. This allele varies from the normal beta-allele by a single nucleotide polymorphism dbSNP Rs334(T;T), where GTG replaces GAG in the sixth codon of the β-globin gene. As a consequence, patients with sickle cell anemia have a mutant form of hemoglobin called HbS (α2βs 2) in their red blood cells. This mutation occurs when a hydrophilic glutamic acid residue (Glu) is replaced by a hydrophobic valine residue (Val) at the sixth position in the β-globin chain.[1]

Hydroxyurea is a powerful substance that stimulates the production of HbF. Extensive research conducted over the last 25 years has provided evidence of its effectiveness in both laboratory and clinical settings for treating SCD in both adults and children. Hydroxyurea acts as a powerful inhibitor of ribonucleotide reductase, an enzyme found in all cells that transforms ribonucleotides into deoxyribonucleotides. Deoxyribonucleotides are essential for the production and repair of DNA. There are several mechanisms of hydroxyurea, including: (1) Stimulating the production of fetal hemoglobin in the red blood cell compartment; (2) Damaging the bone marrow and reducing the number of neutrophils and reticulocytes; (3) Changing the expression of adhesion molecules on circulating neutrophils and reticulocytes, which decreases their stickiness and prevents damage to the blood vessels; (4) Increasing the size of red blood cells and their hydration, which reduces the breakdown of cells and the formation of sickle-shaped cells; (5) Releasing nitric oxide locally, leading to the widening of blood vessels.[2]

Autoimmune hemolytic anemia (AIHA) is a diverse condition that ranges from mild to severe, and is mostly caused by the excessive destruction of a person's own red blood cells (RBC) through various immunological processes. The primary factors involved are autoantibodies, which may or may not include complement (C). However, there is also a growing recognition of other cellular immune effectors, as well as dysregulation of cytokines and inadequate compensation by the bone marrow.[3]

Drug-induced immune hemolytic anemia (IHA) occurs due to several interactions involving a medication, antibodies, and components of red blood cell (RBC) membranes. The three primary processes are the stimulation of autoantibodies, the creation of neoantigens (immune complexes), and the adsorption of drugs onto the red blood cells (RBCs). Immune hemolytic anemia (IHA) related to neoantigen development happens when a medication attaches with low affinity to a normal red blood cell (RBC) component. Consequently, the immune system recognizes the drug-RBC component complex or the structurally modified RBC component as alien. Immune hemolytic anemia (IHA) occurs when antibodies, primarily targeted against a medication, engage with red blood cells (RBCs) that have tightly bonded to the

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drug owing to adsorption. These mechanisms are not mutually independent.[4]

II. CASE REPORT:

A 14 year old female patient was admitted in hospital with the complains of fever since 3-4 days, bodyache, fatigue and nausea vomiting . She was admitted with the almost same complains five times in three months (i.e. between 26 Dec 2023 to 28 March 2023). First time she was admitted on 26 Dec 2023 till 28 Dec 2023 with complains of Fever, Bodyache, Fatigue and had POSITIVE DIRECT COOMBS TEST. [Table 1 and Table 2]The patient was given symptomatic treatment with medication Inj. Ceftriaxone 1gm B.I.D for 3days ,Inj. Amikacin 435 mg for 3 days,Inj. Pantoprazole 26 mg O.D. for 3 days, Inj.Ondansteron 2ml B.I.D for 3 days, Inj.Paracetamol 8.5 ml O.D. for 3days ,Tab Ibuprofen and Paracetamol(Ibugesic Plus) 500 mg T.I.D for 3 days, Inj Pheniramine maleate 0.5 ml/kg/dose and after 1unit of PCV and Cap. Hydroxyurea 500 mg was on hold from last three days. As discharge medication patient was prescribed Tab.Cefixime 200 mg B.I.D for 5 days ,Cap Hydroxyurea 500 mg O.D. for 1 month , Tab. Ibuprofen and paracetamol (Ibugesic Plus) 500 mg for 5 days , Syp. 1 Vita 5ml B.I.D for 1 month, Syp. Neurokind 7.5 ml B.I.D for 1 month ,Syp. Calcimax 5 ml B.I.D for 1 month.

Then patient was admitted on 5 Feb 2023 till 7 Feb 2023 with complains of fever since 3-4 days, Headache since 1 day,1 episode of Vomiting, and lupus at neck region and thigh region with DIRECT POSITIVE COOMBS TEST[Table 3]. Patient was given symptomatic treatment with medication Inj. Amikacin 450 mg T.I.D for 3 days ,Inj. Amoxicillin and Clavulanate (Augmentin) 450 mg T.I.D. for 3 days ,Inj. Pantoprazole 40 mg O.D. for 3 days ,Inj. Paracetamol 4.5 ml on s.o.s basis , Inj Diclofenac 75 mg stat on 7 feb. 2023, Cap Hydroxyurea 500 mg O.D. for 3 days, Syp. 1 Vita 5 ml B.I.D , Tab folic acid 5 mg O.D. , Inj. Tramadol 5 mg stat on 6 Feb 2023, Tab Paracetamol 250 mg stat on 6 Feb 2023.

Patient was shifted to surat civil for further investigation of lupus and for Blood transfusion . Patient was admitted from 7 Feb 2023 to 18 Feb 2023 at surat civil hospital with POSITIVE ANA TEST with suggestion SLE(Systemic Lupus Erthematous) or MCTD(Mixed Connective Tissue Disorder) and was given treatment as Tab. Prednisolone 5 mg O.D. 6 tablets at a time for 4 days, Tab Hydroxychloroquine 200 mg O.D. for 4 days, Inj Cefoperazone Sulbactum 1.5 gm+100 ml for 12 hourly for 12 days ,Inj. Pantoprazole 40 mg 12 hourly , Inj. Ondansteron 2 ml 8 hourly for s.o.s basis, Inj. Paracetamol 100 ml 8 hourly s.o.s if temp exceeds 100, Cap Hydroxyurea 500 mg O.D with 2 units of PCV. With discharge medication of Tab Prednisolone 5mg O.D. 6 tablets at a time , Tab Hydroxychloroquine 200 mg O.D. , Tab. Cefixime 200 mg B.I.D. ,Tab Pantoprazole 40 mg B.I.D, Tab domperidone 10 mg s.o.s for vomiting , Cap hydroxyurea 500 mg O.D.

Patient was again brought to hospital with complains of fever since 2 days , Bodyache since 2 days, nausea and vomiting and generalized weakness and was admitted from 8 March 2023 to 12 march 2023 [Table 4] .Patient was Diagnosed with MCTD with know case of SICKLE CELL DISEASE. Patient was treated symptomatically with medication Inj. Cefoperazone Sulbactum (Zonomax) 1.5 mg B.I.D for 4 days , Inj. Pantoprazole 40 mg B.I.D for 4 days , Inj. Ondansteron 4 mg B.I.D Cap. Hydroxyurea 500 mg B.I.D for 5 days, Tab. Folic acid 5 mg O.D. for 5 days , Inj. Paracetamol 1 gm T.I.D for 4 days , Tab. Ketoralac +Trimethamine (Ketorol DT) 10 mg T.I.D for 5 days , Tab. Prednisolone 5 mg O.D. 6 tablets at time , Tab. Bilastine (Biobil) 20 mg O.D. for 6 days, Syp. Chericoff 10 ml B.I.D for 5 days , Eye drop Moxifloxacin 5ml T.I.D for 5 days, Inj. Tramadol 100mg +100NS TID , Tab. Cefpodoxime (GUD ceff) 200 mg B.I.D after IV antibiotic was stopped . Patient wasdischarged with medication Cap. Hydroxyurea 500 mg O.D., Tab. Folic acid 5 mg O.D., Tab. Ketoralac +Trimethamine (Ketorol DT) 10 mg T.I.D., Prednisolone 5 mg, Syp. Chericoff 10 ml B.I.D., Eye drop Moxifloxacin 5ml T.I.D. Tab. Cefpodoxime (GUD ceff) 200 mg B.I.Dand with 1 unit of PCV

III. DISCUSSION:

With the increasing prevalence of hydroxyurea usage in sickle cell anemia (SCA), it is imperative to have a better understanding of its pharmacokinetics and pharmacodynamics. This is particularly important for young patients, given the rising utilization of hydroxyurea in SCA treatment. Genetic diversity is expected to have a substantial effect on hydroxyurea.It is essential at this step to identify potential genes that have a role in drug absorption, metabolism, clearance, and therapeutic cellular effects.[5] The method by which HU exerts its effects is via inhibiting DNA synthesis while allowing RNA synthesis to proceed.The inhibition

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of ribonucleoside diphosphate reductase limits the conversion of ribonucleotides into deoxyribonucleotides.[2]The main consequences are thrombocytopenia and megaloblastosis, which are thereafter followed by neutropenia and anemia.[6]Moreover, previous research, such as the study conducted by Rosenthal et al., suggests that the swift development of macrocytic anemia in rabbits treated with high doses of hydroxyurea (300–1000 mg/kg/day) may have been caused by hemolysis. Additionally, the enlargement of red blood cells (macrocytosis) could be attributed, at least in part, to the production of abnormally large erythrocytes as a result of intense erythropoietic stress. The presence of hemolysis in our patient is confirmed by the notable reduction in hemoglobin, the increase in LDH and reticulocyte count, and the drop in haptoglobin level. This phenomenon emerged around 30 months into the therapy, endured for a considerable period of time, and ceased upon discontinuation of HU, indicating a possible correlation.[6] There are other forms of hemolytic anemia, including the Warm Antibody Type and the Cold Antibody Type.The three types of autoimmune hemolytic anemia are Mixed Autoimmune Hemolytic Anemia (MAIHA), Paroxysmal Cold Hemoglobinuria, and Drug-Induced Immune Hemolytic Anemia. Our focus is on drug-induced hemolytic anemia, which occurs when a medicine interacts with antibodies and parts of the red blood cell membrane, leading to the development of this condition. The three primary mechanisms involve the binding of drugs to red blood cells (RBCs), the production of neoantigens (immune complexes), and the creation of autoantibodies. Insufficient attachment of a medicine to a normal red blood cell (RBC) component leads to the development of immune hemolytic anemia (IHA) due to the generation of neoantigens. This occurs when the immune system wrongly identifies the drug + RBC component combination or the altered RBC component as foreign. IHA is caused by the drug's adsorption process, wherein antibodies that are primarily targeted against the medication come into touch with red blood cells (RBCs) that the drug has strongly attached to. These processes are not mutually hostile.[4] The clinical presentation and progression of AIHA exhibit significant heterogeneity, ranging from gradual to rapid and with varying levels of anemia. Reticulocytes exhibit a general rise, but indirect hyperbilirubinemia and LDH levels are considerably high, and haptoglobin experiences a reduction. A recent extensive research conducted across many centers found that individuals with coronary artery disease (CAD) had a median hemoglobin level of 9.2 g/dL, with a range of 4.5 to 15.3 g/dL. Among these patients, 36% had a mild form of anemia (hemoglobin levels over 10.0 g/dL), 37% had a moderate form (hemoglobin levels between 8.0 and 10.0 g/dL), and 27% had a severe form (hemoglobin levels below 8.0 g/dL).High levels of LDH are indicative of intravascular hemolysis and hyperacute variants. LDH levels may be inaccurately elevated in instances of tissue necrosis or rapid cell turnover, such as in cases of myocardial infarction, pulmonary embolism, acute hepatitis, or solid and hematological tumors. When discussing hyperbilirubinemia, it is important to note the potential coexistence of Gilbert syndrome and the presence of a related liver condition, where both indirect and direct bilirubin levels are elevated. "[3]".Usually, the administration of therapy that stimulates the production of autoantibodies leads to a positive Direct Antiglobulin Test (DAT) in 11-36% of patients, depending on their dosage, during a period of 3-6 months after the initiation of treatment.The presence of bound IgG is responsible for the positive Direct Antiglobulin Test (DAT), although complement may also be occasionally detected.The peripheral smear test detects the presence of polychromasia, macrocytosis resulting from reticulocytosis, and nucleated red blood cells. Erythrophagocytosis and the presence of microspherocytes are suggestive of autoimmune hemolysis.[4]

According to the Naranjo Adverse Drug Reaction (ADR) Probability Scale, the probability of this patient experiencing Drug-induced Autoimmune Hemolytic Anemia as a rare ADR is classified as PROBABLE. The text is enclosed in the tags.Two case reports were conducted. The first report involved a 59-year-old male patient who was diagnosed with angina and underwent percutaneous transluminal coronary angioplasty. The patient was also diagnosed with thrombocythemia and was treated with acetylsalicylic acid and hydroxyurea, following the local guidelines. After 6 years, the patient reported experiencing fatigue. The physical examination reveals a decrease in hemoglobin (Hb) and red blood cell (RBC) count, as well as an increase in lactate dehydrogenase (LDH), reticulocyte count, red cell distribution width (RDW), and bilirubin levels.A 80-year-old man with a diagnosis of essential Thrombocythemia had treatment with 500mg Hydroxyurea three times a day for 2.5 years. Subsequently, he experienced symptoms of weariness, feverishness, weight loss,

and shortness of breath.The physical examination reveals a reduction in hemoglobin (Hb) and red blood cell (RBC) count, as well as an increase in lactate dehydrogenase (LDH) and reticulocyte count.The peripheral blood smear test involves examining the deformities of red blood cells, which are similar to the deformities observed in our case's smear test, where pieces of red blood cells are present. The laboratory data closely resemble our case, indicating a potential link between the use of hydroxyurea therapy and an uncommon adverse drug reaction known as Drug Induced Autoimmune Hemolytic Anemia.[6][8]Corticosteroids are the initial course of therapy. Oral administration of prednisone at a dosage of 1-1.5 mg/kg/day for a duration of 3-4 weeks frequently leads to an elevation in hemoglobin levels and a decrease in hemolysis in around 70-85% of instances. Subsequently, over a timeframe of about 4-6 months, the administration of steroids should be progressively reduced and discontinued, while continuously monitoring blood counts and hemolytic indices.[3]

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Table:1 Lab parameters of patient from 26/12/22 to 28/12/22

PARAMETERS	26/12/22	27/12/22	NORMAL RANGE
CRP	8.4	-	0-5mg/ml
Temperature	101.2	104.0	97°F-99°F
Hemoglobin	6.5	9.9	12-16 gm/dl
WBC	7600	5700	4000-11000
RBC	2.69	4.0	4.2-5.4 mill
PCV	21.4	32.3	37-47 %
MCV	79.55	80.75	77-93 fl
MCH	24.16	24.75	27-32 pg/ml
MCHC	30.37	30.65	30-36 gm/ml
RDW	21.2	20.7	11-14.5 %
Neutrophil	78	44	40-75 %
Lymphocyte	17	49	20-60 %
Monocyte	04	06	0-10 %
Eosinophil	01	01	0-6 %

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Basophil	00	00	0-1 %
Platelet count	325000	260000	1.5-4.5 lac
Reticulocyte count	-	10.2	0.5%-2.5%
Creatinine serum	0.4	-	0.6-1.4 mg/dl
Blood urea	16.6	-	15-45 mg /dl
Sodium serum	134.0	-	135-145 mmol/ml
Potassium serum	4.4	-	3.5-5.5 mmol/ml
Chloride serum	100	-	96-106 mmol/ml

Table 2: PHERIPHERAl SMEAR TEST

PHERIPHERAL SMEAR TEST	
WBC	Normal
PERIPHERAL SMEAR FOR OPINION (PBF)	
PLT	Adequate
Parasite	Not seen
RBC MORPHOLOGY	
Anisocytosis	++
Poikilocytosis	+
Macrocytosis	OCCASIONAL
Microcytosis	++
Hypochromia	++
Tear Drop Cells	FEW
RBC fragment	+

Table 3:: Lab parameters between 5/2/23 to 7/2/23

PARAMETERS	**5/2/23**	**6/2/23**	**NORMAL RANGE**
CRP	10.1	28.0	0-5mg/ml
Temperature	100.7	98	97°F-99°F

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Hemoglobin	5.4	8.4	12-16 gm/dl
WBC	9200	7200	4000-11000
RBC	2.15	3.11	4.2-5.4 mill
PCV	16.8	27.4	37-47 %
MCV	78.14	88.1	77-93 fl
MCH	25.12	27.01	27-32 pg/ml
MCHC	32.14	30.66	30-36 gm/ml
RDW	28.1	32.8	11-14.5 %
Neutrophil	71	53	40-75 %
Lymphocyte	25	42	20-60 %
Monocyte	02	03	0-10 %
Eosinophil	02	02	0-6 %
Basophil	00	00	0-1 %
Platelet count	254000	228000	1.5-4.5 lac
Creatinine serum	0.3	-	0.6-1.4 mg/dl
Blood urea	19.2	-	15-45 mg /dl
Sodium serum	137.0	-	135-145 mmol/ml
Potassium serum	4.7	-	3.5-5.5 mmol/ml
Chloride serum	104.0	-	96-106 mmol/ml

Table 4: Lab parameters of patient between 8/3/23 to 12//3/23

PARAMETERS	**8/3/23**	**10/3/23**	**11/3/23**	**12/3/23**	**NORMAL RANGE**
D-DIMER	>10000	541.9	-	-	0-500ng/ml
LDH	541.9	97	-	-	135-214U/L
Temperature	97	8.6	98.9	100	97°F-99°F
Hemoglobin	8.6	5700	8.7	9.4	12-16 gm/dl
WBC	5700	3.96	4500	7900	4000-11000
RBC	3.96	29.6	3.85	4.40	4.2-5.4 mill
PCV	29.6	73.74	29.4	31.5	37-47 %
MCV	73.74	21.72	76.36	71.59	77-93 fl
MCH	21.72	29.45	22.6	21.36	27-32 pg/ml
MCHC	29.45	22.2	29.59	29.84	30-36 gm/ml
RDW	22.2	71	21.1	21.4	11-14.5 %
Neutrophil	71	25	61	44	40-75 %
Lymphocyte	25	03	37	53	20-60 %
Monocyte	03	01	01	02	0-10 %
Eosinophil	01	00	01	01	0-6 %
Basophil	00	2740000	00	00	0-1 %
Platelet count	2740000	4.8	206000	258000	1.5-4.5 lac
Reticulocyte count	4.8	0.4	-	-	0.5%-2.5%
Creatinine serum	0.4	13.3	-	-	0.6-1.4 mg/dl
Blood urea	13.3	130.0	-	-	15-45 mg /dl
Sodium serum	130.0	4.5	-	-	135-145 mmol/ml
Potassium serum	4.5	97.0	-	-	3.5-5.5 mmol/ml
Chloride serum	97.0	12.4	-	-	96-106 mmol/ml

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SGPT	12.4	61.0	-	-	0-49U/L
SGOT	61.0	74.2	-	-	0-40U/L
ALP	74.2	0.2	-	-	40-129U/L
Direct Bilirubin	0.2	0.3	-	-	0-0.3 mg/dl
Indirect Bilirubin	0.3	0.5	-	-	0.2-0.8 mg/dl
Total Bilirubin	0.5	9.1	-	-	0.3-1.2 mg/dl
Protein serum	9.1	3.4	-	-	6-8.3g/dl
Albumin serum	3.4	5.7	-	-	3.2-5g/dl
Globulin serum	5.7	-	-	-	2-3.5g/dl