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PRIMARY COLD AGGLUTININ DISEASE IN ADOLESCENT AGE – A RARE PRESENTATION

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ABSTRACT Cold agglutinin disease is a rare form of autoimmune hemolytic anemia caused by cold reacting auto anti bodies directing against the RBC membrane antigens . Majority of cases of Cold agglutinin disease are secondary to infections , autoimmune diseases and Lymphoproliferative disorders . Primary cold agglutinin disease is extremely rare with estimated Incidence of 1 per million and Prevalence of 16 per million with the mean age of presentation being 67 years . A high index of suspicion and meticulous work up is required for diagnosis of primary cold agglutinin disease . Hereby , we aim to discuss a case of Primary cold agglutinin disease presented at adolescent age and it's management

KEYWORDS : Cold agglutinins , Direct Coomb's test , Antibody Titre , Antibody Specificity , Complement Pathway, Anemia ,Immunosupressants.

INTRODUCTION

There are three major types of cold sensitive antibodies producing clinical manifestations : Cold agglutinins causing cold agglutinin disease , Donath Landsteiner antibodies causing Paroxysmal nocturnal Hemoglobinuria and Cryoglobulins causing systemic vasculitis .

1. Cold agglutinins- These are antibodies that recognize antigens on the surface of RBCs at temperatures below the normal body temperature and cause agglutination of RBCs and extravascular hemolysis resulting in anemia , typically without hemoglobinuria . These are typically Ig M Antibodies directed against I or i antigens on the RBC surface .
2. Donath Landsteiner Antibodies – These are typically IgG antibodies that recognize RBC antigens at lower temperatures and bind specifically to P antigen on the RBC surface and cause complement mediated lysis of RBC and not agglutination unlike cold agglutinins. Thus, causing intravascular hemolysis with hemoglobinuria .leading to Paroxysmal nocturnal hemoglobinuria .
3. Cryoglobulins – These are antibodies occurring in association with HCV , autoimmune disorders or monoclonal gammopathy that form immune complexes at lower body temperature without involving RBC and thus lead to systemic vasculitis.

Cold Agglutinin Disease is primarily of two types

1. Primary cold agglutinin disease – That which occurs in absence of an underlying disorder . This is extremely rare with an estimated incidence of 1 per million and an estimated prevalence of 16 per million .The female to male ratio is 2:1 and the mean age at presentation is 67 years with a range from 30 to 92 years. Cold agglutinin AIHA constitutes only 20 to 25% , of all the AIHA cases .
2. Secondary Cold agglutinin disease – That which occurs in the setting of an underlying disease which mostly are infections, autoimmune disorders and lymphoid malignancies .

- Infections – These are the cause of cold agglutinin disease in adolescents and young adults .The mechanism implicated is sharing of antigens on the RBC membrane and the infectious agents. The most common infections implicated are Mycoplasma Pneumoniae and Ebstein Bar Virus . Although there have been cases reported following HIV , rubella , influenza and VZV virus . All the indiviuals having cold agglutinins following infection may not develop the cold agglutinin disease . But , amongst those who develop disease , the symptoms typically occur after two weeks of infection and begin to subside gradually as the infection resolves and typically last for 2 to 3 months .
- Autoimmune disorders – mainly systemic sclerosis and Rheumatoid Arthritis .
- Lymphoid malignancies – Mainly implicated are B cell malignancies like Lymphoma , Waldenstrom Macroglobulinemia , MGUS or unspecified lymphoproliferative disorders .

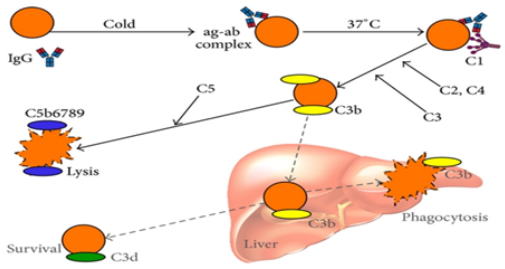
Some Salient Features of cold agglutinins :

- Type – Majority of cold agglutinins are of Ig M subtype . Although IgA and IgG type may also occur .(IgA subtype doesn't cause cold agglutinin disease .)Ig M antibodies are pentameric which places antigen binding sites on RBC far apart to allow a bridge formation in the distance between two RBCs. Thus , multiple RBCs binding to the same IgM molecule is the basis of agglutination by this molecule .
- Light chains- are of kappa type mainly.
- Specificity – is for I or i antigens on the RBC membrane .
- Titre – is the number of dilutions after which the antibody can still cause agglutination . It reflects antibody concentration and avidity . Antibody titre of ≥64 is considered clinically significant .
- Thermal amplitude – The usual temperature range at which the antibodies i.e. cold agglutinins are active is 3 to 4 ◌C .But , many antibodies are active at a higher temperature such as that occurs in the acral areas of the body . This is because , at lower temperatures , the spontaneous brownian movements are reduced , thus strengthening the interaction i.e. weak hydrogen and Van der waals bonds between the antibody and RBC antigens .

Thermal amplitude is defined as the highest temperature range at which the antibody binds to RBC . Most of the clinically significant cold agglutinins have thermal amplitude that exceeds 28° to 30°C.

- Clonality – In primary cold agglutinin disease / those associated with lymphoproliferative disorders , the cold agglutinins are monoclonal in origin and the implicated mutations involve KMT2D and CARD11 genes . While , in cold agglutinin disease following infections / autoimmune disorders , the cold agglutinins are polyclonal in origin . Monoclonal Agglutinins require treatment while the poly clonal antibodies generally do not require treatment and disappear spontaneously once the infection resolves.

Mechanism of hemolysis by cold agglutinins :



IgM Cold agglutinins binds to its cognate Ag , I or i on the surface of RBCs and recruits the components of classical complement pathway such as C1 , C4 and C2 . C1-esterase activates C4 and C2, leading to production of the C3 convertase, which cleaves C3 to C3a and C3b, as illustrated in the figure. C3b-coated RBCs are phagocytosed by macrophages in the reticuloendothelial system (ie, extravascular hemolysis), predominantly Kupffer cells in the liver. Phagocytes tend to engulf the entire cell rather than a portion of the cell membrane, as occurs in phagocytosis mediated by IgG. This may explain the absence of spherocytosis in CAD (or a relatively less impressive spherocytosis), compared with warm autoimmune hemolytic anemia (AIHA). On the remaining circulating RBCs (i.e., those that are not phagocytosed), IgM dissociates upon warming, but C3b remains attached. Surface C3b undergoes cleavage to C3d, which can be detected by the direct antiglobulin (Coombs) test. Complement inhibitors such as CD55 and CD59 on the RBC surface prevent the complement cascade from progressing to the terminal pathway in which the membrane attack complex (MAC; C5b-9) lyses the cells intravascularly. However, when hemolysis is especially brisk (eg, with concomitant infection, surgery, or other inflammatory states), a component of intravascular hemolysis may occur.

Clinical Manifestations of cold agglutinin disease :

It can have either of the two manifestations :

- Features related to cold exposure – Acrocyanosis , Livedo reticularis , Raynaud's phenomenon , cutaneous necrosis/ digital ulceration and pain or discomfort on swallowing cold foods /liquids . All these features reverse on warming.
- Features related to Anemia – Generalized weakness , easy fatiguability , breathlessness on exertion or signs and symptoms suggestive of hyperdynamic state .

Some individuals may also develop jaundice or mild splenomegaly.

Diagnosis of Cold Agglutinin Disease :

Diagnostic Criteria includes the following :

- Evidence of hemolysis (eg, high reticulocyte count, high LDH, high indirect bilirubin, low haptoglobin)
- Positive direct antiglobulin (Coombs) test for C3d only (or, in a minority, C3d plus IgG)
- Cold agglutinin titer of ≥ 64 at 4°C

The accepted threshold for diagnosis is >1:64 and most experts consider a titer above 1:512 to be clinically significant; in many cases, the titer is greater than 1:2048. Most clinically significant cold agglutinins have a thermal amplitude that exceeds 28° to 30°C.

CASE REPORT

A 17 year old female presented to the emergency room of Civil Hospital Ahmedabad on 29/6/19 with chief complaints of generalized weakness, easy fatiguability and breathlessness on exertion since 2 months . She also had associated complaints of yellowish discoloration of urine and sclera , nausea and abdominal pain since 2 months .

She did not have any complaints of pedal edema , chest pain ,palpitations, diarrhea , black stools , decreased urine output or bleeding from any site. Also , there were no complaints fever , arthralgia , skin rashes ,weight loss , abnormal swelling in axillary /inguinal region ,back pain ,cola colored urine or bluish discoloration of toes or fingers on exposure to cold.

She did not have past history of TB, Jaundice, Surgery, Ischemic heart disease , Hypertension, Autoimmune disease or a past history of similar illness . Also there was no history of patient having any viral illness/ pneumonia in the recent past. There was no significant family history .

She had reduced apetite and was on a vegetarian diet . She had adequate sleep and normal bowel and bladder habits . She had no addiction and no history of drug consumption / blood transfusion before the onset of symptoms . Though , she had received 3 blood transfusions at a private hospital in the week prior , before she was admitted at civil hospital , Ahmedabad.

On Examination

Temperature – normal
Pulse – 96/min .
Blood pressure – 110/72 mm Hg
Tongue , Conjunctiva and Nail – Severe Pallor
Sclera – Icterus present
There was no evidence of cyanosis , clubbing , oedema or lymphadenopathy .
Respiratory System – Bilateral Air entry present and no adventitious sounds heard
Cardiovascular System – S 3 was heard . S1 S2 – normal
CNS – conscious, oriented.
Per Abdomen – Soft , non tender and no palpable Hepatomegaly or Splenomegaly.

INVESTIGATIONS

Hemoglobin – 4.10 gm/dL.	(12-15 gm/dL)
WBC – 10.10 $\times 10^3$/cu mm	(4-10 $\times 10^3$/cu mm)
RBC – 0.84 $\times 10^6$ / cu mm	(3.8-4.8 $\times 10^6$/cu mm)
Hematocrit – 12.70%	(36-47%)
MCV – 151 fl	(80-99 fl)
MCH – 49.10 pg	(27-32pg)
MCHC – 32.50 g/dl	(31.5-34.5 g/dl)
Platelets – 328.00 $\times 10^3$/cu mm	
RDW – 9.30	
Polymorphs – 74.00%	(60-80%)
Lymphocytes – 17.00%	(20-40%)
Eosinophils – 3.00%	(1 – 6%)
Monocytes – 6.00%	(2 – 10%)
Basophils – 0.00%	(0.3-1.5%)

Peripheral Smear – Dimorphic Picture predominantly Macrocytic RBC s with few Normocytic Normochromic RBCs. rouleaux formation seen . Moderate anisopoikilocytosis . Macroovalocytes , few spherocytes and polychromatin RBC seen . WBC and Platelets within normal limits . Malarial parasite not seen .

SGPT – 21.10 IU/L (0 – 34 IU/L)
SGOT – 28 .00 IU/L (0-31 IU/L)
ALP – 59 IU/L (58-460 IU/L)
Total Bilirubin – 4.97 mg/dL (0.2-1.2 mg/dL)
Direct Bilirubin – 0.89 mg/dL(0-0.2 mg/dL)
Total Protein – 6.2 mg/dL (6-8.3gm/dL)
Albumin – 3.6 mg/dL (3.2-5 gm/dL)
RBS – 113.8 mg/dL (70-160 mg/dL)
Urea – 34.70 mg/dL (10-45 mg/dL)
Creatinine – 0.72 mg/dL (0.6-1.4 mg/dL)
Sodium – 138 mEq/L (136-145 mEq/L)
Potassium – 3.8 mEq/L (3.5-5.1 mEq/L)
APTT – 29.8 (22.1-36.1)
PT – 11.5 (9.9-12.3)
INR – 1.04
Iron – 155 μg/dL (50-170 μg/dL)
Ferritin – 255 ng/ml (4.63-204 ng/ml)
Vit B12 - > 1500 pg/ml (206-678 pg/ml)
Reticulocyte count (RC)– 18% (0.5-2.5%)
Corrected Reticulocyte count – 7%
Lactate Dehydrogenase(LDH) – 729 U/L (81-234 U/L)
Stool Routine micro – Occult blood positive
Urine Routine micro – NAD
2 AM Urine specimen Routine micro – NAD
Direct Coomb's test – grade 4 +
Indirect Coomb's test – grade 3+
Sickling Test – Negative
Hb Electrophoresis – WNL

Protein Electrophoresis – All major fractions within normal limit . Monoclonal band not seen .
HIV – NR
HbsAg – NR
HCV – NR
ANA – Negative
ANA Profile – Negative
ESR – 2.00 mm .
TSH – 0.8750 μIU/ml (0.38-5.33 μIU/ml)
Free T 3 – 2.00 pg/ml (2.1 -4.4 pg/ml)
Free T 4 – 1.21 ng/dL (0.8-2.7 ng/dL)
MP by card – Negative
Dengue IgM – Negative
Chikungunya IgM – Negative
EBV IgM – Negative
Mycoplasma Pneumoniae IgM – Negative
Sucrose Lysis Test – Negative

Flow Cytometry (PNH Immunophenotyping) – In peripheral blood , 86% neutrophils were gated with CD15 APC and 10% Monocytes were gated with CD45 PerCP.

On Neutrophils , decreased expression of GPI linked antigens CD24 with FLAER was not observed in >3% of gated cells .

On monocytes , decreased expression of GPI linked antigens CD14 with FLAER was not observed in >3% of gated cells .

On Red Blood Cells , decreased expression of GPI linked antigens CD59 was not observed in >3% of cells.

Diagnosis – PNH Clone was not detected .

Advanced Red cell serology report
Antibody screening – Agglutination with C3d .
Antibody Titre – 1:512
Autoantibody Specificity – IgM type

Bone Marrow Biopsy – Normocellular marrow with severe erythroid hyperplasia with normoblastic maturation . Few granulocytic precursors and polymorphs seen . M:E ratio is 1:5.5 (reduced).

ECG – S. tachycardia.
Chest X ray – NAD .

USG (Abdo + Pelvis) – Liver normal in size and echotexture , Spleen 13 cm in size . Rest NAD.

USG for Lymph nodes – No evidence of Lymph node enlargement seen.

Course in the hospital

Patient was admitted at civil hospital Ahmedabad on 29/6/19. Routine investigations were suggestive of Anemia . A high mean corpuscular volume was misleading towards Vit b 12 deficiency as the CBC was not suggestive of pancytopenia . The routine anemia work up done for the patient, was suggestive of indirect hyperbilirubinemia , high Retic count , high LDH , and a positive Direct Coomb's test thus established the diagnosis in favour of hemolytic anemia . By history , the possibility of drug induced hemolytic anemia was ruled out first . Paroxysmal nocturnal hemoglobinuria was ruled out by 2 AM Urine sample screening , Urine for Hemoglobin , sucrose lysis test and Flow cytometry . The possibility of cryoglobulinemia was ruled out from the history , physical examination of the patient not suggestive of any features of vasculitis and HCV negative status. Warm Autoimmune hemolytic anemia was ruled out by the antibody specificity negative for IgG and positive for C3d in the Direct Coomb's test . Further test done with Antibody specific antisera showed a specifity of agglutinins to be of IgM subtype in the titre of 1 :514. A high MCV found in this case is probably spurious due to RBC agglutination analyzed by an automated blood counter . Also , the probable causes for secondary cold agglutinin disease were ruled out by Mycoplasma Pneumoniae IgM , EBV IgM , ANA profile ,USG Abdomen, USG for lymph nodes and Bone marrow biopsy. After all the above enumerated work up , a diagnosis of Primary Cold Agglutinin Disease was established.

Till the diagnosis was confirm , patient was empirically started on Steroids , Methylprednisolone 1 gm diluted in 100 cc NS daily . After 5 days of Methylprednisolone pulse therapy , patient was shifted to oral steroids 1 mg/kg/day slowly tapered off to 0.3 mg/kg/day after one month and then to 0.2mg/kg/day during second month and then 0.1mg/kg/day from the third month ,along with maintenance immuno suppression with azathioprine in a dose of 2mg/kg/day .

Patient was discharged from hospital on 9/7/2019 with oral steroids , azathioprine as mentioned above and rest of the supportive treatment and was called for follow up after 1 month .

CBC on follow up , was suggestive of Hemoglobin of 10.2 gm/dL , RBC 2.43$\times 10^6$ /cu mm . , WBC – 6.60 $\times 10^3$ / cu mm , Platelets – 3.54 $\times 10^3$/cu mm , MCV – 98 fl,

Peripheral smear – Normocytic normochromic and no evidence of any abnormal cells .

This was a rare case of primary cold agglutinin disease , that was responsive to glucocorticoids , most probable reason being agglutinins having a higher thermal amplitude .

Discussion regarding treatment of Cold Agglutinin Disease.

Avoidance of cold is the Primary measure to be undertaken . Warm blankets are to be provided to the patient . Blood products and iv solutions administered should be prewarmed Fever or Infection should be promptly treated . Autoimmune disease if any , should be treated aggressively.

Glucocorticoids and splenectomy are not effective therapy in the majority of patients with CAD, in contrast to warm AIHA

where these therapies are generally very effective . Glucocorticoids may downregulate phagocytosis, but they do not block antibody production. Exceptions may include individuals with IgG cold-reacting antibodies, antibodies with a higher thermal amplitude that cause some warm hemolysis, or mixed warm and cold AIHA. Splenectomy is likely to be ineffective because the liver is the main site of RBC phagocytosis in Cold agglutinin disease unlike that in Warm agglutinin disease wherein , spleen is the main site of RBC phagocytosis.

For Critical hemolysis , when the time taken by immunosupressants to take effect is long and not affordable , Plasmapheresis and IVIG come into rule . Plasmapheresis acts by removing IgM Antibodies in the circulation , but has no effect on the production of IgM agglutinins . The optimal exchange volume and number of exchange procedures is unknown and should be guided by clinical findings and antibody titers; at least one plasma volume per exchange is reasonable. The procedure should be performed in a warm environment and precautions taken to prevent cooling, which could exacerbate cold symptoms and hemolysis. After removal, the half-life for re-accumulation of the IgM is approximately five days. IVIG – There is less experience using IVIG in CAD, and the efficacy has not been well characterized.There have been case reports of it being used in Mycoplasma infections and prior to surgery .

Transfusions — Transfusions should be provided when indicated. Some individuals may have mild disease and may only require transfusions in the setting of severe hemolysis precipitated by infection or during the winter months

Specific B cell directed therapies -

For individuals likely to have a monoclonal cold agglutinin, a specific therapy directed against the clone of B cells or plasma cells producing the antibody is required. Rituximab containing regimens — Rituximabmay be used alone or in combination with other agents. Typical rituximab doses when used as a single agent are based on those used to treat B-cell malignancies (eg, 375 mg/m2 weekly for four weeks) . Lower doses (eg, 100 mg fixed dose weekly for four weeks) have also been used, but with more frequent failures in CAD than in warm AIHA. For those with a specific pre-neoplastic B-cell disorder such as monoclonal gammopathy of undetermined significance (MGUS) or for those in whom a rituximab-containing regimen is ineffective, bortezomib is used.

Rituximab has been used in combination with bendamustine , fludarabine , interferon and steroids .

In case of Rituximab failed regimens, other immuno supressants like Cyclophosphamide, Chlorambucil, Interferon alpha are also tried .

Complement directed therapies are under trial . Complement component C1 is an attractive target because it has enzymatic activity and is upstream of a number of other classical pathway components . C1 consists of three subunits (C1r, C1s, and C1q) Sutimlimab (previously called TNT009) is a humanized monoclonal antibody that targets C1s and has the potential to reduce extravascular hemolysis mediated by C3b. Inhibitors of C1q are also candidate drugs for reducing hemolysis in CAD. Complement protein C3 is another potential target for therapy in CAD. C3 is the point of convergence between all three complement-activating pathways and the initiation of the terminal (lytic) pathway. Therefore, C3 inhibition is expected to block the entire complement system, including the classical pathway and ensuing C3b opsonization, which are the main drivers of hemolysis in CAD. Pegcetacoplan (previously termed APL-2) is a pegylated cyclic peptide inhibitor of C3 that is designed for subcutaneous administration. Anti-complement therapies such as eculizumab, which targets the more distal complement component C5, would not be expected to lessen hemolysis, which is mostly extravascular and mediated by earlier components of the classical complement pathway, although reports have described reduction of hemolysis in selected individuals.Complement directed therapies target the complement mediated lysis and thus prevent extravascular hemolysis but have no effect on the cold induced symptoms. Further , it has been proposed that , Complement directed therapies have to be used for a long period unlike the immunosupressants that have to be used temporarily .

CONCLUSION

In patients of hemolytic anemia, an extensive work up is required to establish the cause of hemolysis . Antibody specificity is must to determine the type of autoimmune hemolytic anemia . Amongst patients with cold sensitive antibodies , it is also necessary to rule out the possibility of Donath Landsteiner antibodies and cryoglobulins before establishing the diagnosis of Cold agglutinin Disease . The diagnosis of Primary Cold agglutinin disease can be established only after completely ruling out the possibility of Lymphoproliferative disorders , infections and autoimmune disorders . Though primary cold agglutinin disease is rare and usually seen in elderly age , it may be exceptionally seen in the adolescent age also.

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