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CASE REPORT
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Cold antibodies association with cellulitis, deep vein thrombosis and abnormal red cells indices: A Case Report.

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ABSTRACT… Objectives: To provide awareness to the pathologists and technologists all about the red cells parameters in cold antibodies concerned cases. **Case Report Findings:** A sixty seven-years-old male admitted in emergency department of our hospital, having clinical history of swelling and pain in both the lower limb and feet, on physical examination, provisionally diagnosed as a case of Cellulitis and deep vein thrombosis(DVT). Blood specimen was obtained for general hematological investigations. Full blood count (FBC) was performed on sysmex XP-100 hematological analyzer which showed invalid findings especially red cells indices which were not corresponding to the hemoglobin (Hb) concentration of the patient. Blood sample was repeated, to confirm invalid red cells indices which showed values as in the 1st blood specimen. Blood smears revealed aggregation of red cells. By warming the ethylenediamine tetra-acetic acid (EDTA) tube containing the blood specimen, in water bath at 37^0C for one hour and repeated the FBC on hematological analyzer and found the corrected red cells indices. **Conclusion:** Basic knowledge of cold antibodies and warming the blood sample at 37^0c for one hour helps the correct diagnosis.

Key words: Deep Vein Thrombosis (DVT), Ethylenediamine Tetra-acetic Acid (EDTA), Full Blood Count (FBC), Hemoglobin.

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INTRODUCTION

Cold antibody disease (CAD) is an autoimmune hemolytic disease in which the antibodies for erythrocyte surface antigens are activated at low temperatures, causing agglutination of red cells. Auto antibodies may be idiopathic or secondary to infection, malignancy, and other autoimmune diseases. CAD is a rare disease and accounts for 15% of all autoimmune hemolytic anemia's. The incidence of CAD is 1 per million people per year.[1] Cold agglutinins can also interfere with laboratory tests. Several case studies have reported that cold antibodies cause erroneous laboratory results, especially in the (FBC) and blood group test.[2-3] In such cases the cold antibody IgM being cold agglutinin's could be detected by just warming the tube at desired temperature (i-e37□c). Cold antibodies are usually immunoglobulin IgM type antibodies which are against the I/i carbohydrate antigens on the surface of red cells. These antibodies are activated at low temperature. This antigen antibody complex leads to activate the classical compliment pathway. When these red cells passes through the reticuloendothelial system, intravascular and extra-vascular haemolysis may occur.[4-5]

CASE REPORT

A sixty seven-years-old male admitted in emergency department of our hospital with presenting complaint of swelling and pain in both lower limb and feet in January' 2019. On physical examination it was provisionally diagnose as a case of (DVT) and Cellulitis which was confirmed by some other reports including colour Doppler study. From our pathological department, we received blood sample for basic routine parameters, biochemistry, coagulation profile and urine analysis including general hematological investigations. FBC was performed

on sysmex xp-100 hematology analyzer which showed invalid findings especially related to red cells indices which were not corresponding with hemoglobin(HB) concentration of patient, Table-I, blood sample 1st. As a routine of our pathological department these results were not released to the concerned consultants. Blood sample was repeated to confirmed invalid parameters of red cells but red cells indices were same as the 1st blood sample Table-I Blood sample 2nd. We observed that blood is sticking to the side of the glass tube. Then we made blood smear on glass slide and on microscopy we found marked red cells agglutination is present in films made at room temperature. At this point cold antibodies was suspected. So EDTA containing blood specimen of patient was incubated in water bath at 37^{O}C for one hour. Blood smear was again prepared by using the May-Grunwald-Giemsa stain and examined microscopically under oil immersion lens it was observed that aggregation of red cells were not present in the blood smear from warmed blood sample. (Table-I Blood sample 2nd A)

SPECIAL SEROLOGIES

For confirmation cold antibodies in the serum of patient we performed some additional tests. Anti human globulin test (Coomb's), both direct and indirect were performed in which direct anti human globulin test was positive while indirect test was found to be negative. Further we measured serum levels of immunoglobulin IgM, IgA, IgG and complement C3, and C4 in the serum of patient. Serum immunoglobulin IgM level was found to be higher than reference limit. Which showed that cold antibodies in this case may be immunoglobulin IgM type, such antibodies usually found in CAD. (Table-II) To avoid these analytic problems we informed concerned consultants and clinical paramedical staff on the importance of immediate analysis of blood specimen of this patient. During the rest of the hospitalization of this patient we performed all blood tests including general hematology by incubating blood specimen in water bath at 37^{O}C for one hour. (Table-III).

Test Name	Blood Sample 1st Before Incubating 37^{0}C	Blood Sample 2nd A Before Incubating 37^{0}C	Blood Sample 2ndA After Incubating 37^{0}C	Unit	Reference Range
WBC	41.5	41.3	40.7	(x10 ^ 3/uL)	4.0 – 10.0
RBC	0.12	0.14	1.73	(x10 ^ 6/uL)	4 .5 – 5.5
Hb	3.6	3.5	3.2	(g/dL)	13.0 – 17.0
Hct	1.5	1.5	12.5	(%)	40- 50
MCV	125.0	125.6	110.2	(fL)	83.0 – 97.2
MCH	300.0	303.0	54.9	(pg)	27.0 – 32.0
MCHC	240.0	245.0	45.8	(g/dl)	31.5 – 34.5
Plt	302	308	297	(x10 ^ 3/L)	150 - 410
Retic.Count	15.0	15.0	15.0	(%)	0.5 – 1.5
Neutrophils	90	90	90	(%)	40 - 80
Lymohocytes	05	05	05	(%)	20 – 40
Monocytes	02	02	02	(%)	02 – 10
Eosinophils	03	03	03	(%)	01 – 06
Nucleated RBCs	03	03	03		/100 WBC's

Table-I. Blood Hematological results before and after incubating at 37^{0}C

Test Name	Unit	Current Result	Reference Range
Serum Complement C3	g/L	0.50	0.8 – 1.6
Serum Complement C4	g/L	<0.01 Rechecked	0.12 – 0.36
Immunoglobulin A (IgA),	g/L	1.18	0.4 – 3.5
Immunoglobulin M (IgM),	g/L	7.19 Rechecked	0.5 – 3
Immunoglobulin G (IgG)	g/L	13.87	6.5 – 16

Table-II. Result of special serologist of the patient

Test Name	2nd Day	3rd Day	Unit	Reference Range
WBC	24.1	14.9	(x10 ^ 3/uL)	4.0 – 10.0
RBC	1.98	1.68	(x10 ^ 6/uL)	4 .5 – 5.5
Hb	5.4	6.9	(g/dL)	13.0 – 17.0
Hct	13.8	18.0	(%)	40- 50
MCV	108.4	107.1	(fL)	83.0 – 97.2
MCH	54.0	41.1	(pg)	27.0 – 32.0
MCHC	45.0	38.3	(g/dl)	31.5 – 34.5
Plt	268	202	(x10 ^ 3/L)	150 - 410
Retic.Count	7.0	7.5	(%)	0.5 – 1.5

Table-III. Hematological results during hospitalization of patient.

DISCUSSION

Cold agglutinins are auto antibodies directed against I/i carbohydrate antigens on the surface of erythrocytes. These are usually IgM types, and rarely, IgG and IgA type antibodies. These antibodies are activated in cold temperature and bind to the I/i antigens on red cells surface. As a result agglutinogen agglutinin complex stimulates the cascade of compliment which results in extra vascular and rarely, intravascular hemolysis.[1] Cold agglutinins may also be found in low titers of the sera of healthy individuals, but they show no activity above 4°C. Pathological cold agglutinins usually react at 28°C to 31°C, or even up to 37°C. The highest temperature at which the antibodies continue to be activated is called the thermal amplitude.[8,14] Clinical presentation and the severity of laboratory findings depend on the degree of thermal amplitude, rather than the antibody titer[1]. Cold antibodies may be monoclonal or polyclonal. Monoclonal antibodies are more commonly seen in idiopathic cases and CAD secondary to lymph proliferative diseases. Polyclonal antibodies are generally determined after mycoplasma pneumonia, Epstein-Barr virus, or cytomegalovirus infections.[2] Circulatory symptoms, such as hemolytic anemia, Reynaud's disease, livedo reticularis, acrocyanosis, and rarely, cutaneous necrosis, which are particularly aggravated during winter, can be seen in CAD.[1] However, the first *suspicious* of CAD is usually a result of a laboratory's inability to measure red cells count and related indices.[8] In hematology analyzer during FBC analysis, cell counts are based on cell diameters. In CAD, antibodies are activated at low temperatures and lead to degeneration and auto agglutination of the erythrocyte membrane. In CAD, there is discrepancy between red cells indices and hemoglobin concentration of patient. Low level of erythrocytes is due to formation of erythrocytes micro aggregates in the patient's blood sample. In hematology analyzer these micro aggregates may count as white blood cells count or single erythrocyte which may be large aggregate or may exclude from total counting which lead to discrepancy between red cell count which result abnormal red cells indices. The hematocrit (HCT) will be spuriously low but mean corpuscular volume (MCV), mean cell hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) values have also been found to be elevated or not measurable.[2,6,17] Platelet counts and indices can also be measured incorrectly, as platelets can also undergo auto agglutination. Other laboratory test that cold agglutinins may hinder is blood group determination. The way to avoid all of these unsuccessful analysis attempts by warming the sample at 37°C before analysis.[8] Agglutination of red cells can visually observed when blood sample is just in EDTA tube and making the blood smears on glass slide and staining by May-Grunvald-Giemsa stain and examining microscopically under oil immersion lens is the best method to confirm aggregation of red cells. By warming the blood sample at 37□c for one hour, all discrepancy between hemoglobin concentration and red cells indices may be removed. These findings are consistent with the results of Nikousedat et al., Ercan S. et al, Kakkar, Yasar and Breuer et al.,[2,6,7,9,10]

Hemoglobin concentrations and WBC's count were un-affected in cold antibodies containing blood sample (Sample 1st Table-I). In cold antibodies there is also difficulty in blood grouping. False blood grouping may be detected which may result life threating condition and death may occur by false transfusion of universal red cells Lodi et al.[12] CAD and cold antibodies are rare. Mostly adults are affected in the 7th decade of life in which female are slightly dominant.[13] FBC may be affected at low temperature due to the presence of crygloblins, which precipitate at temperature between 4□c to 37□c mainly resulting pseudoleukocytosis and pseudothrombocytosis.[15,16] However cold antibodies and cry globulins interfere FBC parameters in different ways but laboratory process for analysis blood sample is same in both cases.

CONCLUSION

Cold antibodies can cause interference in laboratory tests. Informing the laboratory of samples from patients with CAD and ensuring the proper temperature conditions during transportation will lead to accurate results as well as decrease workload and laboratory costs.

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AUTHORSHIP AND CONTRIBUTION DECLARATION

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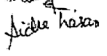
2	Muhammad Faisal Bashir	Statistical analysis Abstact and article writing.	
3	Sidra Ghazanfer	Drafting of article, Collection and assembly of data.	
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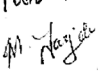
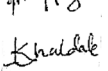
5	Muhammad Tayyab	Proof reading.	
6	Khalid Aftab	Compitation of reseults.	
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