

In Vitro Leukoagglutination: A Rare Hematological Cause of Spurious Leukopenia

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Abstract- Leukopenia secondary to leukocytic agglutination is caused by an ethylene diamine tetra acetic acid (EDTA) which may appear in both benign and malignant states. Ethylene diamine tetra acetic acid induced platelets clumping in peripheral blood has been well established, but invitro leukocytic aggregation is very rarest hematological finding. Pseudo-leukopenia resulting from leukoagglutinins has been reported in the cirrhotic state, infections, autoimmune disorders, uremia, in immunosuppressed state or in various malignancies. Though the condition seems to be benign but very important to be detected as these artifactual findings lead to unnecessary investigations and remarkably changed the overall management plan. Here we report the case of a young patient with this rare finding who was admitted to our hospital with progressive labor pains. The analysis of ethylene diaminetetraacetic acid (EDTA), anticoagulated blood was done on automated hematology analyzer reveals leukopenia. The peripheral smear examination revealed multiple aggregates of leukocytes. On repeat sampling in citrate anticoagulant, the complete blood count showed total leukocytic count of $16.5 \times 10^9/L$ with absolute neutrophilic count of $11.5 \times 10^9/L$. This is a rare case of spurious leukopenia secondary to in-vitro leukocytic agglutination provoked by EDTA anticoagulant.

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Introduction

The phenomenon of in-vitro red blood cells agglutination or platelets aggregation is well established and extensively studied in the literature. Nonetheless, leukocytes agglutination in-vitro is a very rare phenomenon (1,2). Leukoagglutination is associated with spurious leukopenia or may underestimate the leukocytosis (1). This can negatively affect the management decisions in terms of treatment and management. Leukocytes aggregates may comprise of all kinds of white blood cells or may be restricted to one lineage, predominantly to polymorph neutrophils (1). The underlying mechanism of leukocytic agglutination has not been completely clarified, but predominantly it's association with EDTA anticoagulant have been described (1). Leukocyte aggregation caused by an ethylene diamine tetra acetic acid (EDTA) occurs in both benign and malignant disorders. Herein we report the rare case of spurious leucopenia caused by EDTA anticoagulant in full term pregnant female.

Case Report

A 29-year-old female with no prior comorbid, in her third pregnancy, was admitted with labor pains for 6 hours in our hospital. Her past medical and surgical history was unremarkable. In her obstetrical history, she had one live birth and one abortion with normal menstrual history. She was unbooked and denied any antenatal evaluation throughout gestational amenorrhea due to financial constraints. The patient had denied any history of fever or has any active infection. Her general physical examination revealed blood pressure of 130/80 mmHg with a regular heart rate of 96 beats/minute and she was afebrile. Physical examination revealed no lymphadenopathy or hepatosplenomegaly. Systemic examination was unremarkable. Her uterine dimension was appropriate for gestational age with the good progress of labor.

Complete blood count collected in EDTA tube and analyzed by Cell Dyne Ruby (Abbott, USA) revealed hemoglobin level of 9.1 g/dl, hematocrit 27.1%, mean corpuscular volume 88 fl, with total leukocytic count of

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$2.5 \times 10^9/l$ and low normal platelets counts of $124 \times 10^9/l$. Peripheral blood smear revealed interesting finding of copious clumps of 10-15 leukocytes in each field throughout the slide along with the smear edges (Figure1).

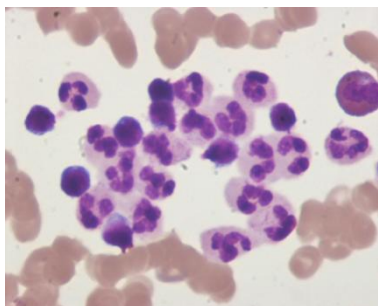


Figure 1. Peripheral blood smear shows leukocytic agglutination induced by ethylene diamine tetra acetic acid (oil emersion x100)

We asked for another blood sample in citrate anticoagulant. A repeat blood sampling in sodium citrate as anticoagulant revealed total leukocytic count of $16.5 \times 10^9/l$ and absolute neutrophils count of $11.5 \times 10^9/l$ with total absence of leukocytes aggregates.

Routine biochemistry investigations revealed serum creatinine of 0.41 mg/dl, urea 29 mg/dl with normal electrolytes levels. Coagulation profile and liver function test were normal. Furthermore, her autoimmune profile was negative, which was done to exclude underlying autoimmune disease process. Her direct Coombs test was also negative.

The patient had delivered vaginally uneventful and discharged to home from the hospital. On follow up visit after 3 weeks, peripheral counts and smear findings were normal; indicating the transient nature of pseudoleukopenia may be elucidated by pregnant status.

Discussion

Leukocytic agglutination in peripheral blood smear is rare transient phenomenon has been accountable since 1983 in literature, and most reports have been based on single cases only (1). It has been delineated in the literature sporadically (2,3). The majority of reported cases have been associated with malignancies, infections, autoimmune disorders or hepatic disorders (3,4). Rarely patients with uremia or in the immunosuppressed state may demonstrate this rare phenomenon (4). We report a 29-year-old female patient who was admitted to the hospital with normal labor. In our case report, no obvious underlying disease process was identified. Leukocyte aggregation is usually a

transient phenomenon, with few cases reported persisting for as long as 5 years (5). The case we reported here was also transient, and the aggregation disappeared after a brief period of 3 weeks.

Even though the accurate reason for leukocytic aggregation *in vitro* has not been elucidated, its relation with EDTA as an anticoagulant has been well described in the literature (1,3,6). Ethylene diamine tetra acetic acid associated agglutination is not unusual for platelets and extensively studied but also has been reported in neutrophils as well as in lymphocytes (4,7). The exact mechanism of this rare phenomenon has not been fully determined (1). Cold agglutinins (IgM) have been implicated as the underlying cause (4). This IgM antibody is directed specifically against the granulocytic antigen and greatly depends on the presence of EDTA anticoagulant. One previous report describes a case of spurious neutropenia caused by EDTA, was reversible after raising the temperature of the sample to 37 C and implicated possible temperature related etiology.

It is also claimed that *in-vitro* leukocytic aggregates are a time-dependent phenomenon (1). The number and size of the agglutinate are dependent on the elapsed time interval between drawing the blood sample into EDTA and preparation of the blood smear and analysis on automated hematology analyzer. Hence this phenomenon is seemed to be EDTA-dependent.

The importance of this false picture is highlighted by the fact, that unrecognized leukoagglutination may prompt to unnecessary treatments such as antibiotic coverage, growth stimulating factors (G-CSF) administration and bone-marrow procedures; however, none of these are valuable nor indicated (4).

Unexplained leukopenia on automated analyzers should always be confirmed by a blood smear examination, which can detect this phenomenon. And subsequently, blood samples should be drawn in container with different anticoagulants such as sodium citrate or heparin. So the accurate leukocyte counts can be obtained.

Though leukocytic agglutination is exceptionally rare in daily practice (2), doctors should be aware of this *in-vitro* phenomenon which may be the underlying cause in pseudo-leukopenia. Recognition of leukoagglutination is most important as the erroneous results may cause unnecessary diagnostic testing and fake diagnoses. Sampling in different anticoagulant will resolve this discrepancy in the total leukocytic count (4).

Spurious neutropenia has no pathological consequence itself. But it can be a vital clue for the possible underlying disease process. It may lead to

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redundant treatments such as antibiotic therapy, G-CSF administration and bone-marrow sampling, none of which are indicated. In conclusion, unexplained leukopenia should always be confirmed by a peripheral smear examination. Sampling in different anticoagulant such as citrate should be under taken in such cases for actual estimation.

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