Abstract:
Objective: To find out frequency, different causes of pseudothrombocytopenia and their further evaluation to reach a final diagnosis
Methodology: This cross-sectional study was conducted at Muhammad Medical Hospital, Mirpurkhas between October 2021 to January 2022. All patients who had an automated platelet count less than 100 were included in the study. While patients with a known cause of thrombocytopenia were excluded. Data was collected with the help of designed proforma. Complete blood count was performed by an automated hematology analyzer. The samples with platelet count less than 100 (flag on platelet counts) were selected for peripheral blood smear review for manual counting to ascertain the cause of thrombocytopenia.
Results: A total of 1800 blood samples were analyzed. 95 patients (5.27%) had isolated platelet count < 100 with no previous history. Peripheral blood smear review was done, 84 patients (88%) had true thrombocytopenia that is low platelet count by both automated and manual techniques. The incidence rate of pseudothrombocytopenia was 0.61%. Clumping due to EDTA was found in 0.27% cases followed by platelet satellitism in 0.05% cases.
Conclusion: Our study reported an incidence of pseudothrombocytopenia to be 0.61%. Further breakdown revealed clumping due to EDTA to be the most common cause. Platelet Satellitism was found to be the cause of pseudothrombocytopenia in 0.05% cases.
Keywords: pseudothrombocytopenia, PTCP, spurious, thrombocytopenia.

Introduction:
Pseudothrombocytopenia (PTCP) also referred to as spurious thrombocytopenia, is an issue that arises during an in vitro specimen-collection process. The phenomenon occurs in the presence of an anticoagulant during the process. This leads to aggregation of platelets (PLTs) and ultimately results in abnormally low PLT counts.1,2 Currently, to improve the diagnostic procedures and monitoring of cell counts in blood samples, the automated hematology analyzers are commonly used in research and clinical laboratories. Clinicians frequently advise peripheral blood smear review for accurate platelet count as it greatly affects diagnosis and provides guidance for effective management of Patients.2 Platelets are anucleate cells that derive from cytoplasmic fragmentation of bone marrow megakaryocytes with a life span of 7-10 days. Normal platelet count is 150-400 x10^9/l. Manual counting, automated by impedance counting/ or light scatter, using immunological markers, and flow cytometry are some of the ways platelet count can be measured.3,4 With the introduction of modern blood analyzers, platelet counts are measured using blood with EDTA as anticoagulant. EDTA is considered the most suitable anticoagulant for cell counting and morphology review because it does not change the morphology of cells within a certain time.
range and it gives fluidity to whole blood samples for electronic particle counting. The disadvantage of EDTA is that it can cause platelet clumping that can give false low results. EDTA irreversibly binds and removes calcium which is required for coagulation cascade. The deficiency of calcium produces conformational changes in the platelet membrane leading to formation of antibodies. These antibodies cross-react with epitopes of platelets glycoprotein IIb/IIIa receptor. This activity unmask hidden platelet receptor epitopes of the fibrinogen receptor GP IIb / IIIa. These epitopes are neoantigens which bind ubiquitous non-pathogenic autoantibodies resulting in aggregate formation leading to Pseudothrombocytopenia. Presence of large or giant platelets is another cause of Pseudothrombocytopenia. Normal platelet size is 2-3 μm in greatest diameter. Giant platelets whose measurement falls out of the upper threshold of automated hem analyzer will be missed in counting leading to false low levels. Another interesting but rare phenomenon responsible for pseudothrombocytopenia is platelet satellitism. Platelets get adhered to polymorphonuclear neutrophils giving them a rosette like presentation. There is only limited data available in local literature therefore, the current study was conducted to evaluate the frequency and causes of pseudothrombocytopenia.

Methodology:
This cross-sectional study was conducted at Muhammad Medical Hospital, Mirpur Khas between October 2021 to January 2022. A non-probability convenience sampling technique was employed for the recruitment of the participants. Ethical approval was obtained from the institutional review board (IRB) prior to the study. Both out door patients and admitted patients were included in this study. All patients who had an automated platelet count less than 100 were included in the study. While patients who had a previous history or have a known cause of thrombocytopenia were excluded from the study. All patients gave consent to be a part of the study. Data was collected with the help of designed proforma. A trained staff collected 5 ml venous blood under aseptic conditions in an EDTA tube by using a sterile syringe. The blood was then received by the hematology laboratory for analysis within two hours.

Blood CP was performed by Sysmex Kx.21 automated hematology analyzer. The samples with platelet count less than 100 (flag on platelet counts) were selected for peripheral blood smear review for manual counting to ascertain the cause of thrombocytopenia. In a well-prepared smear stained with Leishman stain average no of platelets in 10 oil immersion fields was calculated. The average result was multiplied by 15,000 to yield platelet count estimate per ul. Data was presented in tables in form of frequency and percentages analyzed by using Statistical Package for the Social Sciences (SPSS) software computer program version 26.

Results:
A total of 1800 blood samples were analyzed. 95 patients (5.27%) had isolated platelet count < 100 with no previous history. Peripheral blood smear review was done, 84 patients (88%) had true thrombocytopenia. Common causes were infections like malaria, dengue, COVID infections, gestational thrombocytopenia, Immune thrombocytopenic purpura (ITP) and autoimmune disorders. The incidence rate of pseudothrombocytopenia was 0.61% (Table 1). Overall, Platelet Clumps were seen in 814 (45.22%) cases and platelet satellitism around WBC were seen in 188 (10.44%) however, in patients with pseudothrombocytopenia clumps were seen in 0.27% cases while the satellitism was seen in 0.05% cases.

Table No 1: Platelet counts of samples included (n=1800).

<table>
<thead>
<tr>
<th>Platelet Count</th>
<th>No of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 150x10^9/L</td>
<td>195</td>
</tr>
<tr>
<td>150-400x10^9/L</td>
<td>1563</td>
</tr>
<tr>
<td>&gt;400x10^9/L</td>
<td>42</td>
</tr>
</tbody>
</table>

Table No 2: Frequency of isolated thrombocytopenia.

<table>
<thead>
<tr>
<th>No of Patients</th>
<th>True Thrombocytopenia</th>
<th>Pseudothrombocytopenia</th>
</tr>
</thead>
<tbody>
<tr>
<td>95</td>
<td>84 (88%)</td>
<td>11 (11.57%)</td>
</tr>
</tbody>
</table>

Table No 3: Frequency of causes of Pseudothrombocytopenia.

<table>
<thead>
<tr>
<th>Cause</th>
<th>No of Patients</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDTA Clumps</td>
<td>5 (45%)</td>
<td>0.27%</td>
</tr>
<tr>
<td>Giant Platelets</td>
<td>2 (18%)</td>
<td>0.11%</td>
</tr>
<tr>
<td>Platelet Satellitism</td>
<td>1 (9%)</td>
<td>0.05%</td>
</tr>
<tr>
<td>Pre-analytical errors</td>
<td>3 (27%)</td>
<td>0.16%</td>
</tr>
</tbody>
</table>

Discussion:
In current study (11.57%) patients had pseudo thrombocytopenia (PTCP) (Table-2). The most common cause was EDTA PTCP (45%) (Table-3). EDTA induced PTCP was first reported by Gowland et al. Lixia Zhang et al reported the rate of EDTA induced PTCP to be 49.1%. A recent national study conducted in Lahore reported a much higher rate of 75%. EDTA is the number one cause of spurious platelet counts in CBC. In our current study, the satellite pattern of platelets around the WBC are also a cause of pseudo thrombocytopenia, observed in 1 patient (9%) among patients with isolated thrombocytopenia (Table-3).
ardinois et al. reported the prevalence of PTCP as 0.03 - 0.27% in out-patients. It increases up to 15.3% in patients with isolated thrombocytopenia. Giant platelets were another cause of PTCP in 18% of patients in our study. Gayatri Gogoi reported it to be 11.5%. Pre-analytical error was the second most common cause of PTCP in our study (27%). It can be prevented by staff training for proper sampling technique, sample handling and timely testing. Pseudothrombocytopenia (PTCP) is frequently found in laboratory studies, which can result in an error in diagnosis, wrongful treatments and unnecessary testing. PTCP if not diagnosed can result in multiple clinical implications and life-threatening sequelae. Unidentified pseudothrombocytopenia may lead to the performance of further invasive investigations, such as bone marrow biopsy. In some cases, it may also result in the unnecessary postponement of surgeries. Platelet satellitism was the least common cause of pseudo thrombocytopenia in our study. A similar study by Tan et al, reported only 0.1% of cases of pseudo thrombocytopenia due to this cause. On the other hand, a study by et al., reported 10.5% cases of platelet satellitism, with EDTA still being responsible for a large majority of cases (74.4%) However, despite its low prevalence, published literature shows a strong association of platelet satellitism with infections and immunodeficiency, while some studies show that platelet satellitism frequently occurs in healthy individuals, causing pseudo thrombocytopenia. It is highly recommended that when a patient presents with isolated thrombocytopenia with no previous history, a detailed history of presenting symptoms, use of any drugs and family history should be sought. PTCP should always be kept in mind in such cases. A repeat complete count should be done with an alternative anticoagulant such as sodium citrate. Sample should be kept at 37°C to avoid any cold agglutinins. Urgent testing of the sample should be done to avoid time dependent changes. Peripheral blood smear should be made and manual platelet counting should be done to confirm findings.

Conclusion:
It is imperative for the laboratory technicians and clinicians involved to be vigilant in order to address the false data obtained from automated PLT counts. Since PTCP is associated with clinical illness, it requires attention and extensive investigation regarding its process, seasonal variations and other risk factors.

References:


