In incidence of heparin induced thrombocytopenia in hospitalized patients treated with unfractionated heparin in the Azady general hospital

Mohammad A. Khalaf Al Bayatee
Dept. of Medicine, College of Medicine, Tikrit University

Abstract

Heparin induced thrombocytopenia (HIT) is a drug induced immune-mediated syndrome characterized by thrombocytopenia and thrombotic events that may be life-or limb threatening. HIT occurs in up to 5% of patients receiving unfractionated heparin (UFH), and less than 1% of patients receiving LMWH. The aims of the study is to determine the incidence of heparin induced thrombocytopenia in hospitalized patients treated with unfractionated heparin in the Azady general hospital. This study was carried out on 40 patients treated with UFH admitted to the Azady general hospital. Each patient underwent a full clinical evaluation including; disease history, and a physical exam. Blood samples were taken for laboratory tests at base line and every day up to patients discharge, including: EDTA blood for platelet count and blood film, and Serum was obtained from clotted samples for renal and liver function tests. There was mild reduction in platelet count (<30% of base line value) in 27.5% of the patients included in this study. A small proportion 7.5% of the patients showed significant reduction in platelet count (≥30% of base line value) and only one of them (2.5%) progressed to more severe reduction in platelet count (≥50% of base line value) at the 6th day which was labeled as HIT. Stoppage of heparin use in those with significant reduction in platelet count lead to elevation in platelet count within hours up to three days after stopping heparin. All the patients who showed significant reduction in platelet count including the patient with HIT were on IV heparin infusion and none of them were on subcutaneous injection, which is statistically not significant. The cumulative incidence of HIT over all samples was 7% in the first 6 days and even whole follow up period.

Introduction

Heparin induced thrombocytopenia is an adverse drug reaction characterized by thrombocytopenia and high risk for venous or arterial thrombosis. It is caused by heparin dependent platelet activating antibodies that recognize a "self" protein, platelet factor 4 (pf4), bound to heparin. Platelet factor 4, small peptide stored within the alpha granules of platelet, binds to heparin and is released to the blood during treatment with heparin. The resulting platelet activation is associated with increased thrombin generation. Typically platelet count begins to fall between 5 and 10 days after first exposure to heparin.

In subjects with recent exposure, it may occur earlier and, very occasionally, the thrombocytopenia develops after heparin has been discontinued. The condition may complicate heparin administered at any dose, including that used as peri-operative thromboprophylaxis and even when very low doses are used to maintain the patency of intravascular cannulae. Heparin induced thrombocytopenia is around 10 fold less common with low molecular weight preparations than with unfractionated heparin, and has not been reported with synthetic pentasaccharide, and fondaparinux. Heparin induced thrombocytopenia (HIT) should be considered a clinico-pathological syndrome because the diagnosis is based on both clinical and serologic grounds. Thus, (HIT) antibodies seroconversion without thrombo-cytopenia or other clinical sequelae is not considered (HIT),
whereas a diagnosis of (HIT) is made when (HIT) antibody formation is accompanied by an otherwise unexplained platelet count fall (usually ≥ 50 % fall even if the platelet count nadir remain >150×10^9/L), or by skin lesions at heparin injection sites or acute systemic reactions (eg, chills, cardio respiratory distress) after IV heparin bolus administration.\(^{(5)}\)

When (HIT) is diagnosed treatment should started. Management primarily involves principles of Treatment “Six As”
1. Avoid and discontinue all heparin (including LMWH).
2. Administer non-heparin alternative anticoagulant.
3. Anti-PF4/heparin antibody test for confirmation.
4. Avoid platelet transfusion.
5. Await platelet recovery before initiation of warfarin anticoagulation.
6. Assess for lower extremity deep venous thrombosis\(^{(1,6)}\)

**Patients and Methods**

A total of 40 patients on parenteral heparin were included in this study. These patients visited coronary care unite (CCU) at Azadi general hospital and orthopedic department at Azadi general hospital during a period of 16 months (from July 2006- October 2007).

Each patient underwent a detailed history and clinical examination. Of these cases 37 patients presented with ischemic heart diseases (IHD) including myocardial infarction, unstable angina, and angina and 3 patients presented with orthopedic problem (fracture pelvis). The study included 21 (52.5 %) male and 19 (47.5 %) female.

All patients who had an abnormal baseline platelet count (<150 x 10^9/L or >450 x10^9 /L), myeloprolifartive disorder, receiving chemotherapy or radiotherapy or with clinical or laboratory findings compatible with disseminated intravascular coagulopathy (DIC), sepsis, liver cirrhosis, hypersplenism or severe renal insufficiency were excluded from the study.

A total of 60 healthy individuals, sex and age matched, were taken as control group; platelet count was taken for them. The aim was to determine the accuracy of platelet counting procedure.

Four ml of venous blood sample were collected at base line (before heparin administration) and every day until patient discharge, using a clean aseptic venipuncture from the anticubital area of the forearm of each patient and was processed as follows:

1- 2ml of venous blood was put into a tube containing EDTA for platelet count, CBC, and blood film to exclude myeloprolifartive disorders.
2- 2ml of venous blood was put into a plain tube used for estimation of liver function test and renal function test for exclusions of other causes of thrombocytopenia.

**Screening tests:**
Platelet count measurement\(^{(7)}\):
- Principle of the test:
  The blood is mixed with a diluent which preserve both the red cells and the platelets, in which the platelets do not disintegrate or clump even if the diluent suspension is allowed to stand for several hours before the count is made.
- Equipments: Improved neubauer chamber, 0.02 ml pipette, 75 x 10 mm glass or plastic tube, plain capillary tubes, hand counter and test tube rack, petri dish and microscope.
- Reagent:
  Diluent fluid is formal citrate which consists of:
  - 40% formaldehyde → 5 ml
  - Trisodium citrate → 15.6 g.
  - Distilled water → complete to 500 ml.
One in 100 dilution of blood was made by dispensing 0.02 ml blood into 2 ml formal citrate previously placed in a clean test tube. The cell suspension is mixed by hand for at least 2 minutes by tilting the tube through an angle of about 120°.

The modified neubauer chamber was filled by clean glass plain capillary tubes in one action taking care that no fluid flows to the surrounding grooves.

The counting chamber was placed in a moist Petri dish and left untouched for at least 20 minutes, to give time to platelets to settle. The preparation was examined using 4 mm objective and x 10 eye piece in area of 1 mm². The platelets appear under ordinary illumination as small highly refractile particles.

Calculation:

\[
N = \text{number of platelets counted in area of } 1 \text{ mm}^2 (0.1 \mu\text{L in volume}).
\]

\[
\text{Platelets count} = N \times 10 \times \text{dilution} = N \times 10 \times 100 = N \times 1000 \times 10^6 / \text{L}.
\]

Normal value for platelet count in adult\(^8\):

\[
= 150 - 400 \times 10^9 / \text{L}.
\]

Blood film preparation\(^9\):

This was done to exclude microaggregate of platelet that can't be seen by naked eye and can be seen by blood film screening.

Results

The results presented in this chapter were based on the analysis of 40 subjects receiving UFH for variable durations. These subjects were followed up for a maximum of 12 days.

Percent of Maximal Reduction in Platelet Count:

Fig.-1. about 65% of the patients show no change in their platelet count except daily random variations, 27.5% of the patients show mild reduction in their platelet count (<30% of base line value). About 7.5% of the patients showed significant reduction in their platelet count (≥30% of base line value), only one patient of them (2.5%) show reduction in platelet count ≥50% of base line value which is within the definition of HIT, but this not regarded as measure of risk.

Day of Maximal Reduction in Platelet Count:

Fig.-2 shows the day of maximal reduction in platelet count regardless to the percent of reduction. The proportion of subjects that show maximal reduction in platelet within the first 4 days by cumulative percent was 25% of the studied group. Very small proportion of patient show their maximal reduction at 6th day and from (7th to 12th) day post heparin administration which was by cumulative percent only 7.5% of the studied subjects.

Description of the 3 subjects who showed significant reduction in platelets count (≥30% of its baseline value) including the patient with HIT:

Table-1 shows the description of the 3 subjects who showed significant reduction in platelet count (≥30% of its baseline value), including the patient with HIT. As shown all were on IV heparin, two of these three subjects were female, <50 years and had no past history of heparin use. The day of significant reduction in platelet count was the second day and one of them continued to amore severe reduction in platelet count and was labeled as HIT on day 6 and developed thromboembolic complication on day 7.

The relation of stopping heparin and platelet count elevation:

This graph showing the percent reduction in platelet count (compared to its base line value) in the 3 subjects who
showed significant reduction in platelet count. The circle denotes to the day of stopping of heparin administration and shows the relation between stopping use of heparin to the amelioration of reduction in platelet count. As shown the 3 patients show elevation in platelet count within hours up to 3 days of stopping heparin use.

Table (2) shows the risk of developing HIT and significant reduction in platelet count in the overall sample and high risk subset (IV heparin infusion group).

We evaluated the proportion of patients who developed HIT among all those who were treated with UFH, the cumulative frequency of HIT over time was calculated by the Kaplan-Meier technique. For this purpose, patients were censored at the third day following heparin withdrawal. Patients receiving heparin for the whole period of hospitalization were censored on the day of hospital discharge.

This table shows that the cumulative incidence of HIT in the first 6 days was 7% which was equal to the cumulative incidence of HIT in the first 12 days (whole follow up period), this cumulative incidence represent measure of general risk. The same thing for those who show significant reduction in platelet count (≥30% of base line value) their cumulative incidence in the first 2 days was 7% which is equal to their cumulative incidence in the first 12 days (whole follow up period).

Discussion

In this study, the incidence of HIT is reported in 40 patients who received UFH for prophylactic or therapeutic purposes. Daily platelet counts were obtained in all patients. HIT defined as a decrease in the platelet count ≥50% of base line value, beginning five or more days after the start of heparin therapy.\(^{10}\)

The incidence calculated using inception cohort is not a valid measure of risk and is therefore not useful for comparing with different studies. So we use cumulative incidence in the statistical analysis in this study\(^{(11)}\). By the sixth day (and even at the 12\(^{th}\) day) of follow up period, the cumulative risk of HIT in this group of patients reached 7% which is inconsistent with the study of Theodore E. etal (1995, Canada) who found that by the 14\(^{th}\) day of follow up period, the cumulative risk of HIT in the group that he studied was 3.3%.\(^{(10)}\) This difference in the cumulative risk of HIT between our study and Theodor's study may be due to the number of patients in the present study which was very small and further complicated by short duration of treatment and lack of specific antigenic or functional assays to detect the presence of heparin-dependent antibodies.

Concerning the day of developing HIT (keeping in mind that the patient who develop HIT have no past history of heparin use in the last 100 days), the patient showed onset of HIT at the 6\(^{th}\) day which is consistent with the study of John G. and his colleges (April, 26, 2001, Canada) who found that onset of HIT in studied patients (who got no past history of heparin use in the last 100 day) typically occurred between 5 and 10 days \(^{3}\).

Heparin-induced thrombocytopenia is a strong risk factor for thrombotic events. Analysis of the patients in this study showed strong association between HIT and thrombosis. The only patient in the study group who showed HIT developed another attack of MI at the 7\(^{th}\) day post heparin use. This complication may fit the natural history of this patient which was MI or may point out to thromboembolic complications. This complication consistent with the study of Bruno G. and his colleges (December 12, 2002 Germany)\(^{(11)}\) in his study the majority of patients who develop HIT develop arterial thrombosis, but is in consistent with the study of Theodor E.
Incidence of heparin induced thrombocytopenia in hospitalized patients treated with unfractionated heparin in the Azady general hospital

who found that venous thrombosis is more common than arterial thrombosis in those with HIT.\(^{(10)}\)

About 27.5% of the patients included in this study show reduction in platelet count <30% of base line value which is regarded as mild reduction in platelet count and 20% of them occurred in the first 4 days which goes with definition of HIT I\(^{(12)}\).

This finding is consistent with the study of Alison H. and his colleges (1998) who found that only (10%-20%) of patients develop HIT I\(^{(13)}\). This high percent of patients who showed mild reduction in platelet count may be due to that these patients already developed HIT I or may be due to mediation with antihypertensive drugs and/or thiazide diuretic for all of them which are considered as platelet lowering agents, or partly due to that 30% of patients included in the study got past history of heparin use in the last 100 days.

Three subjects from those included in the study showed significant reduction in platelet count (≥30% of base line value) on day 2. One of them continued to more severe reduction platelet count and was labeled as HIT on day 6. The remaining 2 had no chance to develop HIT since heparin was stopped early within the first 3 days. So we can regard them as risk group, and if we analysed their criteria together with the patient who showed HIT we can find many criteria in common, for instance all of them used IV infusion as route of administration and none used sc injection although this finding is statistically insignificant, majority were female and majority were <50 years old and majority got no past history of heparin use.

From analyzing the effect of stopping medication with heparin to the amelioration of the reduction in platelet count we find that all the patients who showed significant reduction in platelet count including the patient with HIT, they showed elevation in platelet count within few hours up to 3 days after stopping heparin use.

The present study conclude that mild reduction in platelet count (<30% of base line value) which shown in 27.5% of the patients may be due to medication with thiazidi diuretic and/or antihypertensive drugs, or may be due to HIT type I.

The cumulative incidence of HIT over all samples was 7% this value was different from other studies due to the number of patients included in this study which was very small, further complicated by short duration of treatment and lack of specific antigenic or functional assays tested for the presence of heparin-dependent antibodies.

References

7-Schmitt BP, Adelman B. Heparin-associated thrombocytopenia: a critical
Incidence of heparin induced thrombocytopenia in hospitalized patients treated with unfractionated heparin in the Azady general hospital


Table (1) Description of the 3 subjects who showed significant reduction in platelets count (≥30% of its baseline value) including the patient with HIT.

<table>
<thead>
<tr>
<th>Subjects with significant reduction in platelets count (≤30%) - including one with HAT</th>
<th>Age in years</th>
<th>Past history of heparin use</th>
<th>Gender</th>
<th>Maximal reduction in platelets count (% of baseline)</th>
<th>Day of maximal reduction in platelets count</th>
<th>Duration of heparin use (days)</th>
<th>Day of significant reduction in platelets count (≥30%)</th>
<th>Day of (HIT) severe reduction in platelets count (≥50%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subject A</td>
<td>62</td>
<td>Positive</td>
<td>Female</td>
<td>-33.8</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>NA</td>
</tr>
<tr>
<td>Subject B</td>
<td>47</td>
<td>-ve</td>
<td>Male</td>
<td>-35.3</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>NA</td>
</tr>
<tr>
<td>Subject C (HAT syndrome)</td>
<td>45</td>
<td>-ve</td>
<td>Female</td>
<td>-68.5</td>
<td>7</td>
<td>6</td>
<td>2</td>
<td>6</td>
</tr>
</tbody>
</table>

Table (2) Kaplan Myer survival analysis estimating the risk of developing HIT and significant reduction in platelets count in the overall sample.

<table>
<thead>
<tr>
<th>Cumulative incidence of outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIT In the first 6 days and whole follow up period (12days)</td>
</tr>
<tr>
<td>Significant reduction in platelets count (≤30%) In the first 2 days and whole follow up period (12days)</td>
</tr>
</tbody>
</table>
Incidence of heparin induced thrombocytopenia in hospitalized patients treated with unfractionated heparin in the Azady general hospital

**Fig (1)** Pie chart showing the frequency distribution by percent of maximal reduction in platelets count compared to its baseline value.

**Fig (2)** Bar chart showing the cumulative frequency distribution of the study sample by day (post heparin inception) of maximal reduction in platelets count.

**Figure (3)** Line graph showing the percent reduction in platelets count (compared to its baseline value) in the 3 subjects who showed significant reduction in platelets count (>30% of its baseline value).