HEPARIN INDUCED THROMBOCYTOPENIA (HIT) SYNDROME IN ADULT PATIENTS AT KENYATTA NATIONAL HOSPITAL – KENYA.

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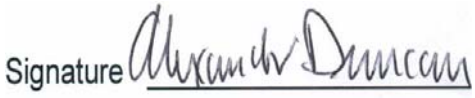

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Dedication

This work is dedicated to my family and to all my close friends.

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Abbreviations

A450	Absorbance at 450 nanometer.
ATIII	Antithrombin III
B.P.	Blood pressure
CATCH	Complication after thrombocytopenia caused by heparin
CCF	Congestive cardiac failure
C.I	Confidence interval
CVA	Cerebrovascular accident
D.M.	Diabetes mellitus
DIC	Disseminated intravascular coagulopathy
DVT	Deep venous thrombosis
EDTA	Ethylene diamine tetra-acetic acid
EIA	Enzyme immunoassay
ELISA	Enzyme linked immunoassay
EQA	External quality assessment
F	Female
HIPA	Heparin induced platelet aggregation
HIA	Heparin induced antibody
HIT	Heparin induced thrombocytopenia
H.I. antibody	Heparin induced antibody.
HIT/T	Heparin induced thrombocytopenia with thrombosis
HIV	Human immunodeficiency virus
HPF4	Heparin platelet factor 4
IQC	Internal quality control
ITP	Immune thrombocytopenic purpura
I.V	Intravenous

KNH	Kenyatta National Hospital
LMWH	Low molecular weight heparin (Enoxaparin)
M	Male
mg	milligram
ml	milliliter
O.D	Optical density
O.R	Odds ratio
ORIF	Open reduction and internal fixation
Q.C	Quality control
Pbf	Peripheral blood film
PF4	Platelet factor 4
PNPP	p-nitrophenyl phosphate
P.R	Pulse rate
PVS	Polyvinyl sulphonate
R.R	Respiratory rate
s.c	subcutaneous
S.D	Standard deviation
SPSS	Statistical package for social sciences
SRA	Serotonin release assay
TTP	Thrombotic thrombocytopenic purpura
U	Units
UFH	Unfractionated heparin (Heparin sodium)
UON	University of Nairobi
USA	United States of America
Yr	Years

Definition of terms

Heparin – is an anticoagulant belonging to the family of glycosaminoglycans, used for prevention and treatment of thromboembolic disorders. Is an anticoagulant of choice when a rapid anticoagulant effect is required[1, 2].

HIT – is an immune mediated complication of heparin therapy caused by the emergence of antibodies that activate platelets in the presence of heparin and is strongly associated with thrombotic complications[1, 2, 3.]

Is defined as platelet count of less than 150 x 10^9/ L or a platelet count drop of > 50% from baseline occurring from day five (5) of heparin therapy and a positive HIA[3]. Can also occur before day five (5) and after day fourteen (14) of heparin therapy (early and late onset HIT).

HIA – is an antibody generated against heparin-PF4 complex, mostly of the IgG type. Antibody: heparin-PF4 complex causes platelet activation leading to thrombocytopenia and thrombosis. It is also associated with skin necrosis and anaphylactic reactions[1, 2, 3.]

Platelet count monitoring – is a primary way of diagnosing HIT. Routine monitoring of platelet count is recommended for patients on heparin therapy including a baseline platelet count before initiating heparin therapy to allow estimation of relative changes[2, 3.]

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ABSTRACT

Background:

Heparin is a commonly used anticoagulant in Kenyatta National Hospital (KNH). Besides bleeding complications heparin induced thrombocytopenia (HIT) with thrombosis, commonly reffered to as HIT syndrome is a well recognized complication of heparin therapy. Some of the patients with HIT may experience other unusual complications such as skin necrosis and anaphylactic reactions which are considered as manifestations of HIT syndrome. HIT antibody with a prevalence of about 8% has been elucidated to be the main pathogenic antibody in the pathophysiology of HIT and skin necrosis, and has been associated with anaphylactic reactions though mechanisms have not been established. There is no documented data on the occurrence of these complications in KNH.

Study design:

This was a cross sectional study.

Objectives:

The study was aimed at screening for the presence of Heparin induced thrombocytopenia (HIT) and its associated unusual complications of skin necrosis and anaphylactic reactions.

Study area:

The study was conducted at Kenyatta National Hospital (KNH) – Nairobi, the major referral hospital in the country and the Haematology and Immunology units of the school of medicine-University of Nairobi.

Study population:

The study involved 188 adults inpatients of both sexes aged 18 years and above on heparin therapy in medical, obstetric, orthopaedic and cardiothoracic surgical wards at Kenyatta National Hospital from April 2009 to August 2009.

Materials and methods:

Data collection was carried out through clinical history taking and physical examination of the patients, review of patient's medical records, and laboratory tests (total blood count by automated cell counter – cell Dyn 1300, peripheral blood film and H.I. antibody detection by ELISA – *ZYMUTEST HIA IgG*). All the information was entered in a questionnaire. Data analysis was aided by a computer (SPSS version 15), Chi-square test and Fisher's exact test were used

for correlation between variables and a p-value of < 0.05 was considered to yield a statistical significant result.

Results:

Of 22,592 adult inpatients admitted to the KNH in the period of April to August 2009 a total of 294 patients on heparin were recruited, and of these 188 patients were followed up for thrombocytopenia, skin necrosis and anaphylactic reactions.

The majority of study patients (58%) were female and (42%) were male. The highest proportions (16.5%) of the patient were aged between 30-34 years while the age bracket 55-59 years had the least number of patients (3.7%). A large proportion of patients were from the medical ward (59%). Most of the patients (72.90%) were on UFH (heparin sodium) preparation.

The overall prevalence of HIT was 2.70% (3.65% with UFH and 0% with LMWH). All the 5 (2.7%) of the study patients who had HIT were female, from the medical unit and were all on UFH preparation. There was no statistically significant difference in the HIT status between – the two heparin preparations, UFH and LMWH (enoxaparin) (p-value 0.167); and the different patient populations (p-value 0.313).

Heparin induced skin necrosis and anaphylactic reactions were not seen in any of the study patients that were followed up for a total of seven (7) or ten (10) days.

Of the total study patients, the overall prevalence of heparin induced antibody which is associated with the risk of developing these complications was 17%, (18.20% with UFH and 13.70% with LMWH). Medical ward patients had the highest frequency of antibody formation (21.60%) with the least in obstetric patients (5.90%). There was no statistically significant difference in the heparin induced antibody positivity between - the two heparin preparations, UFH and LMWH (p-value 0.463) and between the different patient categories (p-value 0.214).

Conclusion

This study shows that Heparin induced thrombocytopenia (HIT) syndrome is rare in KNH. Heparin induced thrombocytopenia (HIT) was found at a low prevalence of 2.7%, and appeared to occur mainly in medical patients and in those on UFH preparation.

The unusual complications of HIT (skin necrosis and anaphylactic reactions) are uncommon at KNH.

Heparin induced antibody (HIA) which is associated with the risk of developing these complications was found at a low prevalence (17%), and appear to predominate in medical patients and in those who were on UFH preparation. However as the validation of the HIT antibody test and some of the steps in the interpretation of the HIT antibody test (inhibition of a positive result by high concentration of heparin) were not performed, there is a probability that the prevalence of this antibody could be much lower.

With the low prevalence of HIT and the shortcomings of this study, routine laboratory screening for detection of the HIT antibody may not be justified, further studies will need to be done.

Recommendations

Although the study shows that HIT, skin necrosis and anaphylactic reactions are rare in KNH it is still advised to perform a close clinical and laboratory surveillance of platelet count in all patients on therapeutic or prophylactic doses of UFH.

Further studies especially in various patient population-specific groups are needed for a proper approximation of the prevalence of these complications.

More advanced studies on HIT are recommended which will involve adequate laboratory investigations to exclude other causes of thrombocytopenia, measures to confirm thrombosis (Doppler ultrasonography, venography) and probably longer duration of follow up to enable proper approximation of the prevalence of HIT and skin necrosis.

BACKGROUND AND LITERATURE REVIEW

Heparin has been a widely used anticoagulant for the treatment and prevention of venous and arterial thromboembolic disorders in medical and surgical patients since 1950's.[1]

It is a glycosaminoglycan composed of chains of alternating residues of D-glucosamine and an uronic acid. Its molecular weight ranges from 5000 to 30000.[2]

The anticoagulant effect of heparin is mediated largely through its interaction with antithrombin III (ATIII); this produces a conformational change in ATIII and therefore markedly accelerates its ability to inactivate the coagulation enzyme thrombin (factor IIa), factor Xa, and factor IXa.[2]

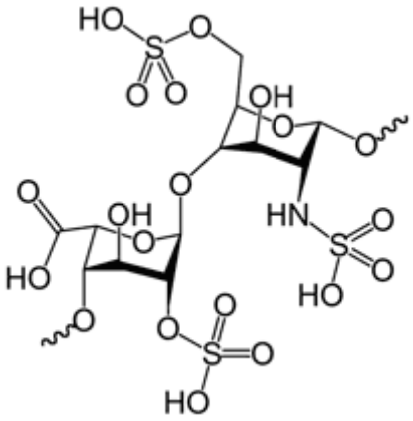


Figure 1: Chemical structure of Heparin (alternating chains of N-acetyl-D-glucosamine and D-glucoronic acid)[4]

Heparin is derived from porcine gut or bovine lung. There are two types of heparin: the standard unfractionated heparin (UFH) and its derivative low molecular weight heparin (LMWH). The low molecular weight heparins (LMWH) are produced by chemical or enzymatic degradation of UFH and have a molecular weight of less than 8000.[2]

Both types of heparin are available at Kenyatta National Hospital (KNH). Unfractionated heparin (UFH) is available as either heparin sodium or heparin sulphate. Enoxaparin is the LMWH in use at KNH under a trade name of clexane.

Clinical uses of heparin include prevention and treatment of venous and arterial thrombosis, pulmonary embolism, and deep venous thrombosis. It is also used for early treatment of patients with acute myocardial infarction and unstable angina; in patients undergoing cardiac

bypass surgery and for flushing of intravenous catheters in intensive care units and renal settings.[2]

There are a number of complications associated with the use of heparin including bleeding, thrombocytopenia (HIT), anaphylactic reactions, skin necrosis, osteoporosis, hypoaldosteronism, priapism and hepatotoxicity [2.]

Heparin induced thrombocytopenia (HIT) syndrome is a well recognized and serious side effect of heparin coming second to bleeding complications. It is immunologically mediated and the HIT antibody has been elucidated to be the main pathogenic antibody in the pathogenesis of HIT. Paradoxically some patients with heparin induced thrombocytopenia (HIT) develop arterial or venous thrombosis associated with variable but relevant rates of morbidity and mortality. Heparin can therefore cause the same devastating complications that it is meant to prevent. Skin necrosis occurring at the site of heparin injection and anaphylactic reactions occurring shortly after heparin injection are considered as unusual complications of the HIT syndrome[3].

Heparin induced thrombocytopenia with thrombosis was described in 1958 by Weismann and Tobin in 10 patients who developed emboli while on treatment with heparin. This complication was described as the white clot syndrome due to the appearance of white platelet thrombi occluding the lumen of blood vessels. Six of the patients died of recurrent multiple arterial emboli.[5]

There are no documented studies on the prevalence of these complications (HIT, skin necrosis and anaphylactic reactions) in Africa; most studies have been conducted in Europe and North America. The protocol for platelet count monitoring in patients on heparin is lacking in KNH and laboratory testing for heparin induced antibody which is associated with these complications is also not done. The occurrence of these complications may therefore be under- or over diagnosed.

HIT is defined as a decrease in platelet count following exposure to heparin. [3] There are two categories of HIT a benign form also called type I and an immune mediated form called type II.

It has been proposed that the term “HIT type I” be changed to “nonimmune heparin associated thrombocytopenia” and the term “HIT type II” be changed to “HIT” to avoid confusion between the two syndromes. In accordance with these new recommendations, in this study the term HIT refers strictly to the immune mediated type II form.[3]

Benign non-immune heparin associated thrombocytopenia: it affects up to 10% of all patients receiving heparin. It is caused by direct platelet activation with heparin, occurs earlier after heparin therapy initiation with platelet count decreasing rapidly within the first two days. The platelet count usually remains above 100,000/µl and will return to normal within one to five days despite continued heparin administration. This type of thrombocytopenia is not associated with increased risk of thrombosis and does not require treatment.[3]

HIT (immune mediated form): it typically presents 5 to 10 days after the initiation of heparin and is often associated with platelet counts of less than 100 x 10/L. it is an immune mediated adverse reaction and is associated with thromboembolic sequela in a variable proportion of patients[3, 6]. The clinical manifestation may however occur before day 5 and after day 14 (early and late onset HIT).

Incidence of HIT

The overall frequency of HIT is difficult to precisely define because the risk is associated with the type of heparin, study design and most importantly the clinical situation.[6]

The incidence of HIT at Kenyatta National Hospital (KNH) is unknown as there are no published studies on HIT within this region and most parts of Africa. Most of the studies reviewed here have been conducted in USA, Canada and Europe.

In general the incidence of HIT is greater with bovine versus porcine heparin, with UFH versus LMWH, and in post-surgical (cardiac > orthopaedic > vascular > general) versus medical and obstetric patients.

The first study investigating the incidence of thrombocytopenia during heparin therapy was reported by Bell et al in 1976. The incidence of thrombocytopenia (platelet count less than 100 x 10/L) was found to be 30.7%. Subsequent studies found that the incidence of heparin induced thrombocytopenia was lower than this initial report particularly with the recognition of the immune mechanisms of thrombocytopenia.[7]

Warkentin et al conducted a prospective study in the year 2000 comparing incidence of HIT antibodies, thrombocytopenia and thrombosis between cardiothoracic and orthopedic surgery patients, as well as UFH versus LMWH. The incidence of HIT antibodies in cardiac surgery patients (all on UFH) was 50% while that of HIT (thrombocytopenia) was 1%. The incidence of HIT antibodies in orthopedic surgery patients was 14.1% (with UFH) and 7.5% (with LMWH);

while that of HIT (thrombocytopenia) was 4.9% (in both UFH &LMWH). Among these patients (cardiac &orthopedic surgery) with HIT (thrombocytopenia) 60% developed thrombosis.[8]

An analysis of studies conducted from 1995 – 2005 indicates a higher frequency of HIT antibody formation in cardiac surgery patients (range 25 – 64%). Despite this high frequency the incidence of thrombocytopenia and thrombosis are much lower than in orthopedic surgery patients in which the frequency of antibody formation is much lower (7.8 – 14.1%).[8,9]

Lindhoff-last et al conducted a multicentre prospective trial in 2002 involving 1137 medical patients assigned to therapy with UFH and LMWH for the treatment of DVT. This study found a significantly higher incidence of HIT antibodies in UFH group than in the LMWH group (20.7% vs 7.5%). Although the frequency of HIT (thrombocytopenia) was similar for both UFH and LMWH groups (0.53%) the incidence of thrombosis was higher in the former and absent in the latter (50% vs 0%).[10]

The incidence of antibody formation in medical patients in one large retrospective study (10,348 patients) by Shuster et al was found to be 1%, with an even lower frequency of thrombocytopenia (0.5%).[1]

In another prospective study by Harbrecht et al, neurological patients receiving UFH demonstrated a high incidence of antibody formation (20.5%) and thrombocytopenia (2.5%) with 80% of HIT patients developing thrombosis.[11] However similar patients treated with LMWH demonstrated a much lower incidence of antibody formation (1.8%) with no sequelae of thrombocytopenia and thrombosis.[12]

Preliminary results from the CATCH registry (Complication After Thrombocytopenia Caused by Heparin), indicate an incidence of HIT in 0.2% in patients who have received heparin for more than 96 hours and 2.1% for patients receiving heparin in coronary care units. The final results of this study which aims to clarify the incidence evaluation and outcome of HIT in over 7000 patients is keenly awaited.[13]

In a retrospective cohort comparative study of pregnant and non-pregnant women Fausett et al found no cases of HIT in the pregnant group. An incidence of 4.1% was however found in the non-pregnant cohort. In a retrospective study of LMWH safety in pregnancy Lepercq et al demonstrated no cases of HIT among 624 pregnancies. Data from these studies suggests that HIT is rare in pregnancy.[14, 15]

A well designed prospective randomized study has been reported in literature by Warkentin et al in 1995 involving 665 orthopedic patients and comparing the incidence of HIT with standard UFH and LMWH. The frequency of heparin induced antibodies was 7.8% with UFH and 2.2% with LMWH. HIT developed in 2.7% of patients with UFH, none of the patients on LMWH developed HIT.[9]

Etches et al conducted a prospective pilot study to determine the incidence of HIT in a paediatric intensive care unit population exposed to heparin. Three of the 233 study patients developed heparin induced antibody giving an incidence of 1.3% for antibody formation with none developing thrombocytopenia or thrombosis. All three patients were post cardiovascular surgery patients.[16]

In a 2 year retrospective analysis of suspected HIT cases in a tertiary paediatric hospital, Newall et al identified 4 cases; 2 of the patients had cardiopulmonary bypass surgery and only one patient out of the three investigated had heparin induced antibodies. With an average of 29 children exposed daily to heparin in the study institutions, this incidence was much lower than expected.[17]

Pathophysiology of HIT – HIT is immunologically mediated. The principal antigen is a complex of heparin and platelet factor 4 (PF4). Platelet factor 4 (PF4) is a chemokine of the C-x-C family, a small positively charged molecule of uncertain biological function normally synthesized in the bone marrow megakaryocytes and stored in platelets within the α granules which is released by activated platelets.[18, 19, 20.]

The binding of heparin to PF4 induces a conformational change in both molecules exposing several antigenic epitopes of the PF4 molecule. The ratio of heparin to PF4 is critical for constitution of the antigenic complex (optimal ratio ranging from 1.4 to 1.6). Heparin affinity for PF4 depends upon molecular weight, chain length, and its degree of sulphation which explains the differences in incidence of HIT observed with different heparins.[21]

The conformational change leads to generation of heparin-PF4 antibodies most frequently of IgG type. However antibodies of IgM and IgA class have been demonstrated in some studies.[22]

The complexes of Heparin-PF4 form on the surface of activated platelet where they are subsequently recognized by HIT antibodies. The antibodies bind to the Heparin-PF4 complexes on platelets via their Fab portion. The Fc portion of the HIT antibody then binds to platelet Fc

receptor (FcγRIIA) leading to further platelet activation. Increased expression and polymorphism in the platelet FcγRIIA receptors is responsible for the risk to develop HIT.[23]

Thrombocytopenia in HIT is largely due to the clearance of activated clumped platelets and antibody coated platelets by the reticuloendothelial system.[24]

Activated platelets also release prothrombotic platelet derived microparticles. These microparticles promote excessive thrombin generation frequently resulting in thrombosis. The HIT antibody- HPF4 complexes also interact with monocytes leading to tissue factor production. HIT is also associated with direct endothelial damage through binding of excess PF4 to heparan sulphate found on endothelial cells the complex is then recognized by HIT antibody resulting in immune mediated vascular injury. Both of these latter processes may contribute further to the activation of the coagulation cascade and thrombin generation.[25]

Clinical features of HIT - Thrombocytopenia and thrombosis are the predominant clinical features of HIT. Despite the thrombocytopenia, bleeding complications are uncommon. Although thrombosis occurs in a small fraction of heparin treated patients, when it occurs it tends to be extensive with greatly increased morbidity and mortality.[3]

Thrombocytopenia occurs in 90% of patients with immune HIT. Typically the thrombocytopenia is of moderate severity with median platelet count of approximately 50 – 70 $x10^9$/L in patients with HIT. Very few have platelet count of less than 15 x 10^9/L. Ironically these patients are at an even greater risk of thrombosis compared to patients with less severe thrombocytopenia.[7]

Thrombocytopenia by standard definition is based on a fall in platelet count below 150 x 10^9/L, but this definition has been found to be inappropriate for HIT because of the existence of clinical situation where the platelet count may not fall below the lower limit of the reference.[26] In about 10% of patients with HIT the platelet count never falls below 150 x 10^9/L. This may be because HIT complicates a post operative course that is characterized by thrombocytosis and occasionally because of chronic thrombocytosis.[26]

An improved definition for thrombocytopenia - a fall in platelet count of 50% or greater from the baseline count is now used in diagnosing HIT clinically.[27]

This improved definition has superior operating characteristic and identified many more patients with HIT while retaining the same high specificity as observed with the standard definition.[27]

Timing of thrombocytopenia: In patients receiving heparin for the first time thrombocytopenia in HIT becomes clinically apparent 4 – 20 days after initiation of heparin, most commonly between days 5 and 12 with a median on day 10.[28]

In patients who have received heparin in the past and were previously sensitized, platelet count may decrease within the first 3 days, or even hours of re-exposure to heparin.[28]

The median time to platelet recovery (>150 $x10^9$/L) is 4 days after discontinuation of heparin. Some patients may take up to two weeks and longer particularly when thrombocytopenia is severe.[7]

The antibody disappears within 2 – 3 months after cessation of heparin therapy.

Thrombosis: Is the most important complication of HIT. Both arterial and venous thromboembolic complications are seen in HIT patients. Heparin induced thrombocytopenia and associated thrombosis has been reported despite normal platelet counts. However most thrombotic cases have occurred when the platelet counts was decreased by at least 30% to 50%.[28]

This thrombosis often involves occlusion of large vessels particularly the distal aorta and femoral arteries. The thrombosis may lead to stroke, myocardial infarction, limb artery occlusion requiring amputation, deep venous thrombosis and pulmonary embolism. Also noted are mesenteric infarction, renal artery thrombosis, and adrenal infarction with subsequent acute or chronic adrenal insufficiency.[29] Consequently abdominal angina or limb pain can be a warning sign of incipient HIT.[30]

Venous thrombosis appears more likely in postoperative HIT patients and arterial thrombosis seems more likely in HIT patients with preexisting cardiovascular disease or recent cardiovascular surgery.[28]

The diagnosis of HIT remains a clinical one supported by a confirmatory laboratory testing. The "4T's" of HIT (Thrombocytopenia, Timing, Thrombosis, and absence of oTher causes of thrombocytopenia) has been recommended by to be used to assess patients with clinical suspicion of HIT.[30]

Laboratory testing of HIT - Two types of laboratory tests are available for establishing a diagnosis of HIT: Functional assays and Immunoassays.

Functional assays: - are tests based on platelet aggregation or activation. They include Heparin induced platelet aggregation (HIPA) assay, the Serotonin release assay (SRA) and flow cytometric assays that detect platelet microparticle release.

SRA – This test is regarded as 'Gold standard' for laboratory diagnosis. The principle of this test is based on release of ^{14}C serotonin by washed platelets at therapeutic concentrations and inhibition with very high concentration of heparin. A release of more than 20% of ^{14}C serotonin at low levels of heparin which is abrogated at high dose heparin in a two point assay is indicative of a positive result. The SRA is 95% sensitive and specific. The SRA requires radioactive markers and expensive sophisticated equipment.[31, 32]

Immunoassays – have been developed for detection of heparin induced antibodies. The first enzyme linked immunosorbent assay (ELISA) was developed in 1995 using macromolecular complexes of H-PF4 as the target antigen immobilized to a solid phase.[33] Commercial assays have subsequently been developed.Two PF4-dependant antigen assays have been developed and are commercially available for detecting HIT antibodies.

One assay known as ASSERACHROM (Diagnostica Stago, France) detect antibodies reactive to H-PF4 complexes coated on plastic micro well strips. H-PF4 antibodies are then detected by peroxidase labeled goat antihuman IgG, IgA and IgM antibodies. The final absorbance cut off value can thus be determined using a positive reference sample. The test is easy to perform and can be done in any laboratory with access to multichannel pipettes plate washing equipment and ELISA plate reader.[34]

GTI-PF4 assay – the propensity for polyanionic substance other than heparin to expose cryptic antigenic sites on PF4 led to the development of EIA that detect antibodies reactive to PF4 bound to polyvinylsalphonate (PVS). The bound anti H-PF4 antibodies are detected by peroxidase labeled goat antihuman antibodies as in the Asserachrom assay.[34]

The advantage of this assay is that the ratio of PF4 to PVS is not critical (unlike that for H-PF4 complexes) and secondly the antigen complex is stable for longer periods of time.[34]

Immunoassays are easier to perform and have high sensitivity (80 – 100%) but low specificity. The lower specificity of ELISA may be attributed to the presence of low titre naturally occurring antibodies with crossreactivity to H-PF4 complexes or to the low cut offs values recommended by manufacturers.[35] No single assay has 100% sensitivity and specificity, although testing

becomes most effective when functional and immunoassays are done in combination and multiple samples are taken.

Management of HIT - The most essential element in the treatment of HIT and HIT/T remains discontinuation of all heparin including heparin flushes, subcutaneous heparin and heparin coated indwelling catheters. [36]

The heparin derivative (LMWH) cannot be used in patients with HIT because of the strong crossreactivity of the HIT antibody with the LMWH-PF4 complex.[36]

Currently three non-heparin anticoagulants that do not cross react with HIT antibodies are available for alternative anticoagulation in HIT. These include Danaparoid (Orgaran) and direct thrombin inhibitors Lepirudin (Recombinant Hirudin) and Argatroban.[36]

Additional treatment considerations: aspirin is beneficial in vitro and may have some clinical benefit. Platelet glycoprotein IIb/IIIa inhibitors reduce thrombin generation indirectly and inhibit platelet aggregation. Prostacyclin analogues act as natural vasodilators and inhibit platelet aggregation. However these agents lack direct anticoagulant effects and do not inhibit Fc receptor-mediated activation of platelets by HIT antibody. Hence these agents should not be used as frontline treatment in HIT.[36]

Heparin induced skin necrosis:

Is a rare complication, and is considered a manifestation of Heparin induced thrombocytopenia and thrombosis syndrome though most patients who develop skin necrosis do not have thrombocytopenia.[37]

Incidence, clinical presentation and diagnosis - In a ten- year retrospective study of HIT by Warkentin et al it was noted that 10 – 20% of patients who develop HIT antibodies during heparin therapy develop skin lesions at the site of heparin injection. The lesions range from painful erythematous plaques to frank skin necrosis.[37]

Warkentin et al observed six patients over a 30- month period (1993 -1995) who developed heparin induced skin lesions. Four of the patients developed painful erythematous plaques; the other two patients developed skin necrosis. All six patients were receiving standard UFH for post operative antithrombotic prophylaxis. All six patients' sera exhibited strong heparin induced IgG antibodies. Only two of the six patients developed thrombocytopenia. [38]

Fureder et al compared features of patients with UFH- and LMWH- induced skin necrosis between 1987 – 1998 (UFH – 15 patients; LMWH – 7 patients). In both groups they were of similar age (36 – 87years); there was no difference in the time of onset of skin lesions from start of heparin treatment (approximately one week in both patient groups); heparin induced antibodies were detected in two patients on LMWH and all the patients on UFH. Thrombocytopenia seems to be more common in patients with UFH induced skin necrosis. Almost all patients who had LMWH induced skin necrosis had previous exposure to heparin treatment.[39]

Tietge et al reported a case of LMWH induced skin necrosis occurring a distance from the injection sites and without thrombocytopenia. This male patient was receiving prophylactic LMWH subcutaneously into the abdominal wall and developed skin necrosis on the 16th day in both thighs.[40]

Pathophysiology – is due to ischaemia of the skin secondary to thrombosis of small vessels caused by aggregation of platelets in these vessels. This suggests type II hypersensitivity reaction resembling HIT. Another mechanism is that of immune mediated vascultis resembling a type III immune complex mediated reaction.[41]

Management – it is recommended to stop all types of heparin, switching to alternative anticoagulant, and wound care. Most wounds heal spontaneously, but some may require surgical debridement and sometimes skin grafting.

Heparin induced anaphylactic reactions:

Anaphylaxis to heparin is rare, it occurs with both UFH and LMWH.[38, 42] It is also considered to be a manifestation of the HIT syndrome. The mechanisms underlying these reactions have not been elucidated, though most patients are found to have circulating HIT antibodies in serum.[43]

Incidence and clinical presentation – the incidence of heparin induced anaphylactic reactions is not known but is rare and most of the literature is in the form of case reports.

Warkentin et al conducted a nested cohort study comparing frequency of unusual clinical sequelae in postsurgical patients on heparin prophylaxis (20 patients positive for HIT antibody and control 80 patients negative for HIT antibody). Of the 20 patients positive for HIT antibody four (4) developed skin necrosis and one (1) developed anaphylactic reaction following an intravenous heparin administration. It occurred 10 minutes after heparin infusion and manifested

as chills, rigors, dyspnoea and tachypnoea. None of the patients in the control group presented with these complications.[43]

Berkun et al reported a case of a patient with renal failure who developed anaphylaxis after receiving heparin during haemodialysis. He presented with urticaria, angioedema and bronchospasm[44]

Tiu et al reported a case of a woman with infected leg haematoma who developed skin necrosis with LMWH (enoxaparin). LMWH was stopped and was switched to intravenous UFH. Two minutes after the infusion she developed shortness of breath which progressed to cardiorespiratory arrest.[45]

Smith et al described anaphylactic reaction to LMWH (enoxaparin) in a patient with acute ischaemic heart disease. The patient developed marked tongue swelling 30 minutes after subcutaneous injection of enoxaparin. This progressed to bradycardia and severe chest pain.[42]

Diagnosis – is usually confirmed by clinical presentation, skin tests and elevated serum tryptase levels.[42, 44]

Management – it is recommended to stop heparin therapy, switching to alternative anticoagulant and giving injection hydrocortisone, adrenalin and cardiopulmonary resuscitation

RATIONALE

Heparin induced thrombocytopenia (HIT) and its manifestation skin necrosis and anaphylactic reactions are well recognized heparin associated complications after bleeding. They are uncommon with the incidence of HIT ranging from 1% to 5%, skin necrosis occurring in 20% of those who are positive for heparin induced antibody, and incidence of anaphylactic reactions not documented but reported to be much rarer. Though they are uncommon, with the dramatic increase in the use and indications for heparin it is likely that the number of people affected is increasing. All the three complications have been associated with heparin induced antibody.[9, 37, 41,]

HIT is associated with the risk of serious thromboembolic complications such as stroke, myocardial infarction and limb ischaemia resulting in amputation. Without treatment mortality in HIT patients is about 20 – 30% with equal morbidity caused by arterial and venous thrombosis.[3] Skin necrosis carries a high morbidity (with some of the lesions requiring skin grafting) and occasional mortality. Some anaphylactic reactions may be fatal (e.g. anaphylactic shock). The significance of these complications is further reflected by the fact that some issues related to management are still unclear especially the lack of an ideal alternative anticoagulant.

Following the first description of HIT in 1958, there have been over 1000 publications in the last 50 years on clinical presentation, pathophysiology, laboratory tests and treatment. Currently most studies have concentrated on convenient laboratory tests and ideal treatment of HIT. There are no many studies documented in literature on skin necrosis and anaphylactic reactions, most of the literature is in the form of case reports.

There are no documented studies on the prevalence of these complications in Africa; most studies have been conducted in Europe and North America. The protocol for platelet count monitoring in patients on heparin is lacking in KNH and laboratory testing for heparin induced antibody which is associated with these complications is also not done. The occurrence of these complications may therefore be under- or over diagnosed.

Laboratory confirmation of these complications by detection of Heparin induced antibody (HIA) and proper platelet count monitoring will improve the management of patients with these life threatening complications by enabling early intervention (i.e. stopping heparin therapy, switching to an alternative anticoagulant and preventing re-exposure to heparin for those already

sensitized). This will help in preventing morbidity and mortality associated with these complications.

This study was aimed at screening for the presence of heparin induced thrombocytopenia (HIT) and its unusual manifestations skin necrosis and anaphylactic reactions. It was hoped that the results of this study would enlighten medical practitioners on the existence of the complications and encourage monitoring for thrombocytopenia, skin necrosis and anaphylactic reactions in patients on heparin therapy. Study findings would also form the basis of justification on whether to or not to introduce the laboratory tests for detection of H.I. antibody.

RESEARCH QUESTION AND OBJECTIVES

Research question

Is there Heparin induced thrombocytopenia (HIT) syndrome in adult patients at KNH?

Broad objective

To screen for the presence of heparin induced thrombocytopenia (HIT) syndrome in adult patients on heparin therapy at Kenyatta National Hospital.

Specific objectives

1) To determine the presence of heparin induced thrombocytopenia (HIT) in adult patients on heparin therapy at KNH.
2) To establish the presence of heparin induced skin necrosis in adult patients on heparin therapy at KNH.
3) To establish the presence of heparin associated anaphylactic reactions in adult patients on heparin therapy at KNH.
4) To detect the presence of heparin induced antibodies in adult patients on heparin therapy at KNH.

MATERIALS AND METHODS

a) **Study design**: This was a cross-sectional study conducted at Kenyatta National Hospital (KNH); Haematology unit and Immunology unit –school of medicine, University of Nairobi.

b) **Study population**: The study included 188 adult inpatients of both sexes, aged 18 years and above who were on heparin therapy at Kenyatta National Hospital (KNH), Nairobi from April 2009 to August 2009. This is a referral hospital and represents the general population in the city.

c) **Inclusion criteria**.

Adult inpatients of both sexes aged 18years and above on heparin therapy and has given consent to participate in the study.

Exclusion criteria.

Adult inpatients of both sexes aged 18 years and above on heparin but found to have:

- A low baseline platelet count (<150 x 10^9 /L) or platelet count more than 600 x 10^9 /L.
- Clinical or laboratory findings suggestive of other causes of thrombocytopenia:
    - *Increased platelet destruction*
        - Non immune - septicaemia, DIC and TTP, infections such as malaria and HIV.
        - Immune – ITP, post transfusion purpura, drug induced (quinine, rifampicin)
    - *Decreased platelet production* – cytotoxic drugs, leukaemia, aplastic anaemia, bone marrow infiltration diseases.
    - *Others* – hypersplenism.
- History of drug/ food allergy or bronchial asthma.
- Declined consent to participate in the study.

d) Sample size: the sample size was 188 patients. This was obtained using the descriptive study sample size formula.

$$n = \frac{Z^2\alpha/2\ P(1-P)}{d^2} = \frac{1.96^2 \times 0.08(1-0.08)}{0.04^2} = \textbf{\textit{180.}}$$

Where $Z^2\alpha/2$ - Is a standard normal deviate at 95% CI; $\alpha = 0.05$.

P - Prevalence of the disease =0.08 (8%) – prevalence of HIT antibodies adopted from a randomized prospective study in Canada by Warkentin et al (1995).[9]

d - degree of precision at 0.04 (4%).

n - sample size

e) Recruitment and obtaining consent of study patients

Study patients regardless of gender were recruited from the four wards: cardiothoracic surgical ward, orthopedic surgical ward, medical and obstetric wards.

Cardiothoracic surgical ward – recruitment was done on operation days (every Tuesday and Thursday). All patients were screened.

Orthopedic surgical wards – surgeries are conducted daily, recruitment was done on Tuesday, Thursday and Friday (the two days were selected by a simple randomized technique), all patients were screened.

Medical wards – consecutive recruitment, all admitted patients on heparin were screened, any day.

Obstetric wards – consecutive recruitment, all admitted patients on heparin were screened, any day.

Screening of patients for fulfillment of inclusion and exclusion criteria – this was done through clinical history taking from the patients, physical examination, review of the patients' medical records and laboratory tests. Laboratory tests included those from patients' medical records and

the baseline full blood picture where platelet counts and other parameters were used to screen study patients.

Consent – those who fulfilled the inclusion and exclusion criteria were informed about the study (the advantages and disadvantages of the study and how the results shall be communicated to them) and consent was obtained from them. Written informed consent forms were signed by those who agreed to participate in the study. (see appendix 1).

f) The type of data collected

Patient's characteristics – age, sex, ward, and diagnosis.

Heparin therapy information – indication, preparation, dosage and route.

Number of patients with complications – HIT, skin necrosis and anaphylactic reactions.

g) Data collection procedure - Methods of data collection

- Administration of a questionnaire - all the data collected from each patient was filled in a questionnaire; this included patient's characteristics, information on heparin therapy and complications. The questionnaire consisted of three parts: part A – information from patient's files; part B – laboratory tests and part C – physical examination (See appendix 2).

- Laboratory tests – blood samples were collected for full blood picture (total blood count and peripheral blood film) and for Heparin induced antibody (HIA) detection.

- Physical examination – for detection of skin necrosis, signs of anaphylactic reactions and thrombotic complications of HIT. This was done by the principal investigator and by research assistants (intern doctors of the ward). This was done consecutively from initiation of heparin therapy up to the 10^{th} day.

    - Skin necrosis – examination at the heparin injection sites as well as on intravenous infusion sites was done. Mainly looking for painful erythematous plaques and frank skin necrosis.

    - Anaphylactic reactions - physical examination was done looking for signs of urticaria, angioedema (e.g. swollen tongue), and signs of shock (cold extremities, sweating, hypotension – B.P < 90/60 mmHg, tachycardia – P.R > 100/minute).

Systemic examination – respiratory system examination for dyspnoea, tachypnoea (R.R > 24/minute). All these usually develop within minutes after heparin therapy injection or infusion.

- Complications of HIT (Thrombotic events) – general and systemic examination for abdominal and lower limbs tenderness or oedema.

- Clinical history taking and review of patient's medical records –

  - For any reported symptoms of anaphylactic reactions (breathlessness, chest pain, bronchospasms and urticaria).

  - For any warning signs of thrombosis (abdominal and lower limb pain).

h) Specimen collection, transport and storage.

- Blood sample for full blood picture – two (2)ml of venous blood was drawn from each patient under aseptic techniques and collected into a sterile, labeled vacutainer with EDTA anticoagulant. This was done before initiation of heparin therapy, then on 3rd, 7th and 10th day. The sample was analyzed for total blood count and a peripheral blood film prepared on the same day of collection.

- Blood sample for detection of Heparin induced antibody - four (4)ml of venous blood was drawn under aseptic techniques and was collected in a well labeled, sterile plain vacutainer without anticoagulant. This was done on the seventh (7th) or tenth day (10th) from initiation of heparin therapy.

  The samples were transported to the immunology laboratory (UON). Transportation of the samples from the wards to the laboratory: samples were kept in a container with ice blocks (to maintain temperature at 2 - 8°c). At the laboratory they were kept at 2-8°c for 24 hours, after which serum was harvested and put into a properly labeled cryovials and stored at -20°c until analysis.

i) Specimen analysis

Total blood count – was performed by an automated cell counter (cell Dyn – 1300) and for each sample a peripheral blood film was done for morphology assessment of platelets and other cells. Reporting of the blood films was done by the principal investigator under supervision by one of the supervisors (Haematologist).This was done at the haematology laboratory (UON).

Heparin induced antibody detection - was done by a qualitative ELISA – *ZYMUTEST HIA IgG.* This was done by the principle investigator under supervision by one of the supervisors at the Immunology laboratory (UON). The samples and the ELISA kit were brought to room temperature and tests for detection of Heparin dependent antibodies (anti-H:PF4 IgG) were carried out following the instructions as per the kit manufacturer.

The assay principle: the diluted assayed serum sample is introduced into one of the coated plate, and supplemented with a platelet lysate. When present, the heparin-dependant antibodies, of the IgG isotype, form complexes on to the biologically available unfractionated heparin, immobilized and saturated. Following a washing step, bound antibodies are revealed with the immunoconjugate, which is made of goat polyclonal antibodies anti-human IgG (Fcγ specific)-peroxidase conjugate. This immunoconjugate reacts specifically with IgG isotypes. Following a new washing step, the peroxide substrate, Tetramethylbenzidine (TMB) in the presence of hydrogen peroxide is introduced and a blue colour develops. The colour turns yellow when the reaction is stopped with sulfuric acid. The colour developed is directly proportional to the amount of heparin-dependant antibodies, of the IgG isotype, present in the tested sample. (For details of the assay procedure see appendix 3).

Interpretation of the results was based on the absorbance at 450nm (A450) values, and were expressed as positive or negative.

- Positive: A450 > 0.50
- Negative A450 $\leq$ 0.50

Limitations of the assay

- As for any autoantibody assay, clinical situation such as presence of inflammation, infectious diseases, autoimmune diseases, immune complexes, can induce a high

background, which can be within the weak positive range. It is therefore recommended to check for the possible presence of antibodies on another specimen collected later.

- Erroneous results can occur from bacterial contamination of the test materials, inadequate incubation periods, inadequate washing or decanting of test wells, exposure of substrate to stray light, omission of test reagents, exposure to higher or lower than prescribed temperature requirements or omission of steps.
- Although a positive reaction obtained in this assay may indicate the presence of heparin associated antibody, the detection of such antibodies however does not confim the diagnosis of HIT.
- Some patients may have naturally occurring antibodies to PF4 or other chemokines.

N.B: the ELISA is a qualitative screening method for HIA

Serotonin release assay (SRA) is the gold standard for HIA detection, though not easily available in most centres.

In this kit optical density values of > 0.3 to < 0.5 were indicated as weak negatives, and were treated as negatives to take care of the false positive reactions.

For further quantitative interpretation of the optical densities see appendix 5.

Quality Assurance – H.I. Antibody detection

Pre-analytical:

Properly labelled blood samples were collected without anticoagulant, under aseptic techniques and the same number was used to identify the sample through all analytical steps. Haemolyzed and lipaemic samples were discarded. Serum was aliquoted into properly labelled cryovials and stored at - 20°c until analysis.

Analytical:

The laboratory numbers of the specimen were counterchecked to ensure correspondence, the ELISA kit was stored at 2-8°c, further verification of the kit was done to ensure that it has correct reagents and have not expired. Proper equipment was used as instructed.

Assay procedure was carried out as per instructions by the kit's manufacturer, including usage of correct volumes and dilutions, observing the incubation times and avoidance of light in the incubation steps. All other cautions were adhered to as instructed by the kit's manufacturer.

Positive and negative controls were used in every batch analysis of the specimens and were treated in the same manner as the patient samples.

Post-analytical:

Expected absorbance values for positive and negative controls were used to ensure that the results obtained are valid.

Cut-off values for positivity and negativity were followed as per the instructions by the kit's manufacturer, to ensure correct interpretation of the results.

Recording of the results to the questionnaire was carefully done, ensuring correspondence between assigned laboratory number and the correct patient.

Quality Assurance - Full blood picture.

Internal quality control (IQC).

Pre-analytical:

- Blood samples were collected under aseptic techniques.
- Collection was done into a sterile, EDTA anticoagulant containing vacutainer while ensuring blood does not clot.
- Correct labelling was done.
- Transport to the laboratory for analysis was done soon after collection.

Analytical:

Sample analysis for total blood count and preparation of a peripheral blood film was done on the same day of collection.

Procedure of quality control for Total blood count (cell Dyn - 1300)

- Controls were removed from the refrigerator while initializing the machine and allow them to warm at room temperature for at least 30 minutes with constant mixing on a roller.
- Low, normal and high controls were then run before any patient specimen was run.
- Results given by the machine were compared with the expected results for each control given by the manufacturer.
- When the results were out of range, corrective measures were followed –this included checking the instrumentation: performing cleaning or priming cycles and checking for blocking of tubings etc; checking if the QC material is viable (not expired); the IQC was then re-run again.
- Levey Jennings charts were plotted after every week and general performance of the machine assessed. Values were valid when within +/- 2 SD units.
- Reference ranges that were age and sex adjusted for each parameter were entered into the machine.

Procedure of quality control for peripheral blood film (Pbf).

- The quality of staining was checked after every batch of 30 films.
- Inspection of the slide under the light microscopy was done for control differentiation.
- The results of the films were interpreted according to the expected standard results (lymphocytes – blue, monocytes – grey blue, neutrophils – pink to orange, and platelets – purple).

Post-analytical:

- Proper recording of the results into questionnaires was done to ensure that there is correspondence between the assigned laboratory number and the correct patient.
- Calculations for percentage of platelet count drop were carefully done and information filled into the questionnaire for correct interpretation of thrombocytopenia.

External quality assessment (EQA) procedure for Total blood count (cell Dyn – 1300)

- The UON participates in the proficiency testing programme run by the National health laboratories – South Africa for University laboratories in Africa managed by the World Health Organization.

ETHICAL CONSIDERATION

Authority was sought from Kenyatta National Hospital and University of Nairobi (KNH-UON) Ethical and Research committee (see appendix 6 & 7).

The study was undertaken after formal approval by the committee.

Informed consent was obtained from participating patients.

Confidentiality of the participating patients was maintained. The name of the patient appeared on the questionnaire for the purpose of follow up only.

Results of the test were communicated to the attending medical practitioner for further appropriate intervention and management. As analysis for H.I. antibody detection was run in batches, diagnosis of heparin induced thrombocytopenia was made initially by the characteristic platelet count drop (the attending clinician was advised to stop heparin therapy and switch to alternative anticoagulant) while awaiting laboratory confirmation.

DATA ANALYSIS

The data collected were entered into a study questionnaire, then into a computer database from which spreadsheets were made and transferred to the SPSS (version 15) statistical software for analysis.

Description of various variables was expressed in proportions, percentages, frequency distributions in the form of tables and graphs.

Multivariate analysis was done for correlation between variables. Chi-square test and Fisher's exact test were used to detect any significant correlation between different variables. A p-value of < 0.05 was considered to yield a statistically significant result. Odds ratio was calculated for relative risk estimation.

RESULTS

Of 22,592 adult inpatients in KNH (from April to August 2009), a total of 294 study patients on heparin were recruited from the four wards (medical, obstetric, orthopaedic and cardiothoracic surgical wards). Of the 294 study patients, 188 (64%) were successfully screened for complications of heparin therapy (HIT, skin necrosis and anaphylactic reactions). The rest 106 (36%) were dropped from the study due to various reasons (discharge or death before day five (5) of heparin therapy, abnormal baseline full blood picture and specimen unsuitability – lipaemic, haemolyzed specimens).

Heparin induced thrombocytopenia (HIT) was found among the study patients with a prevalence of 2.7% (5 / 188), skin necrosis and anaphylactic reactions were not observed.

Heparin induced antibody (HIA) which is associated with these early heparin complications was found in 17% (32 / 188) of the study patients.

Demographic characteristics of the study patients

A total of 188 patients on heparin therapy were recruited in the study. Of these, 109 (58%) were female and 79 (42%) were male. The age bracket of 30-34 years had the highest frequency of study patients (16.5%) while age bracket 55-59 had the least frequency (3.7%). The mean age was 41.66 years with a standard deviation of 15.5. The median age was 38 years. The youngest patient was 18 years and the oldest was 80 (Table 1).

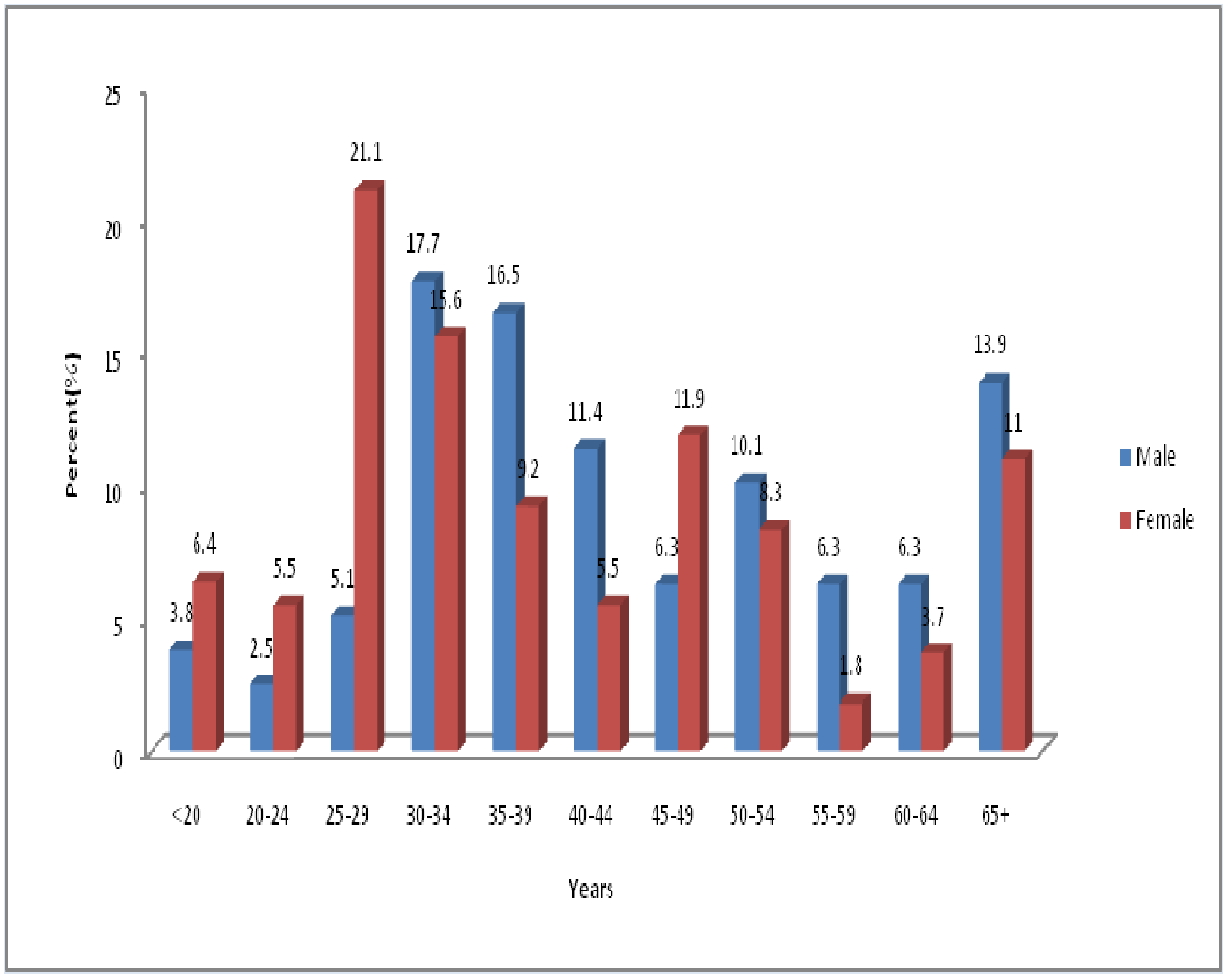
Table 1: Demographic characteristics of the study patients, n = 188.

Characteristic		n (%)
Sex	Male	79 (42)
	Female	109 (58)
Age group	< 20	10 (5.3)
	20-24	8 (4.3)
	25-29	27 (14.4)
	30-34	31 (16.5)
	35-39	23 (12.2)
	40-44	15 (8)
	45-49	18 (9.6)
	50-54	17 (9)
	55-59	7 (3.7)
	60-64	9 (4.8)
	≥ 65	23 (12.2)

Age and sex distribution of the study patients

Of the 188 study patients, 79 (42%) were male and 109 (58%) were female. The male age bracket of 30-34 years had the highest frequency of study patients (17.7%), while 20-24 years had the least frequency (2.5%). The female age bracket with highest frequency of study patients (21.1%) was 25-29 years, while the least frequency (1.8%) was in the age bracket of 55-59 years (Figure 2).

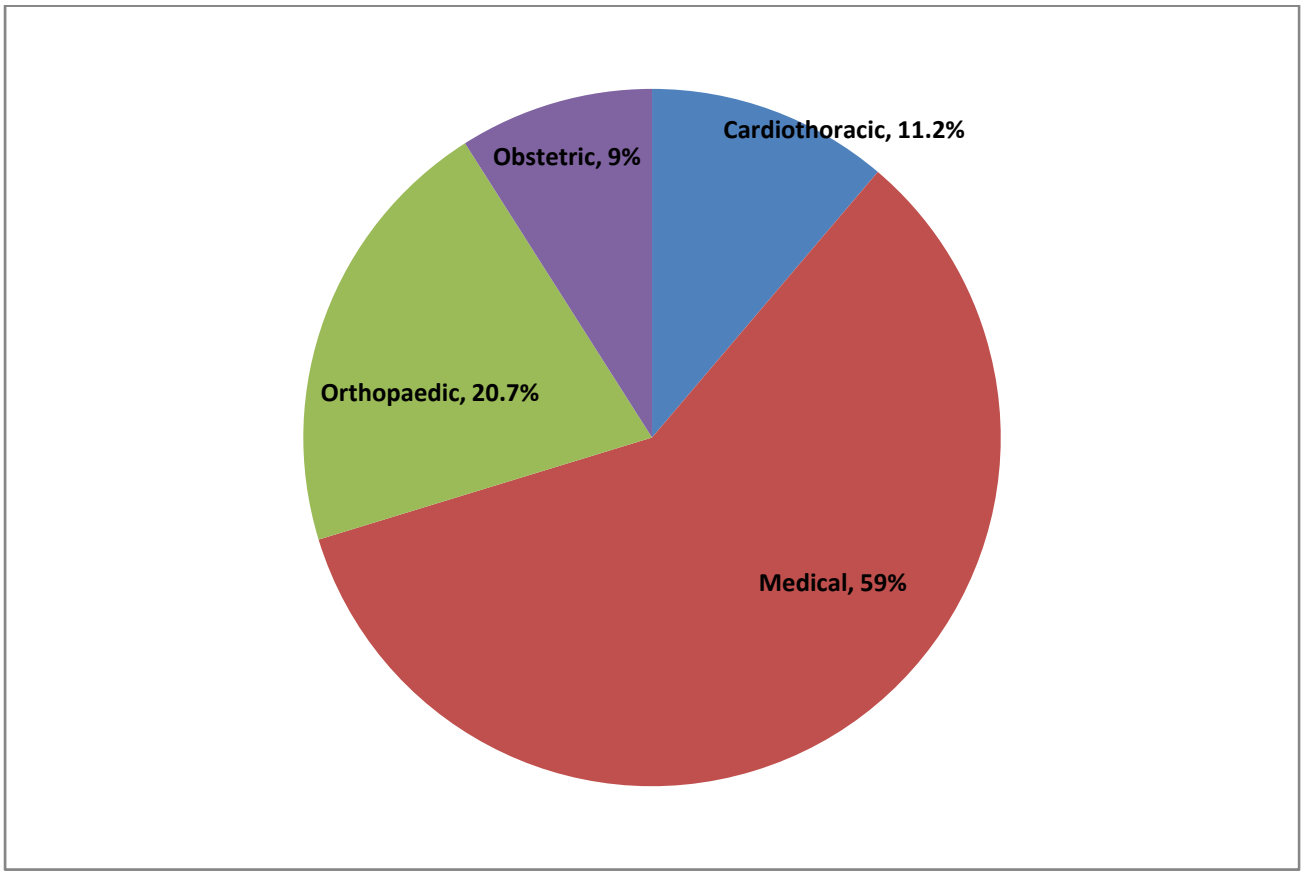
Figure 2: Age and sex distribution of the study patients (n =188)



Distribution of study patients by wards

Of the 188 study patients, the majority, 111 (59%) were from medical wards, 39 (20.7%) from orthopedic surgical wards, 21 (11.2%) from cardiothoracic surgical ward and the least number of patients were from obstetric wards 17 (9%). (Figure 3)

Figure 3: Distribution of study patients by wards, n =188.



Indications for heparin therapy in various patient groups

Of the 188 study patients 159 (84.6%) were on heparin for prophylactic reasons and 29 (15.4%) for therapeutic reasons. Of the 29 patients on heparin for therapeutic reasons, 18 patients were from medical ward - for treatment of DVT (15), P.E (2), and cardioembolic stroke (1); eleven (11) patients were from obstetric ward for treatment of DVT in pregnancy.

Majority of the patients who were on heparin for prophylactic purposes were from medical ward (93) and were on heparin for various medical reasons (stroke, CCF, D.M, hypertension etc). Thirty nine (39) were on heparin for orthopedic surgery- related prophylaxis (ORIF of the tibia and femur, hip replacement surgeries and spinal injuries). Twenty one (21) were from cardiothoracic ward and were on heparin for cardiac bypass surgery- related prophylaxis (valve replacement and coronary artery bypass graft). The rest (6) were on heparin for various obstetric reasons (preeclampsia, valvular disease and post valve replacement in pregnancy). (Table 2).

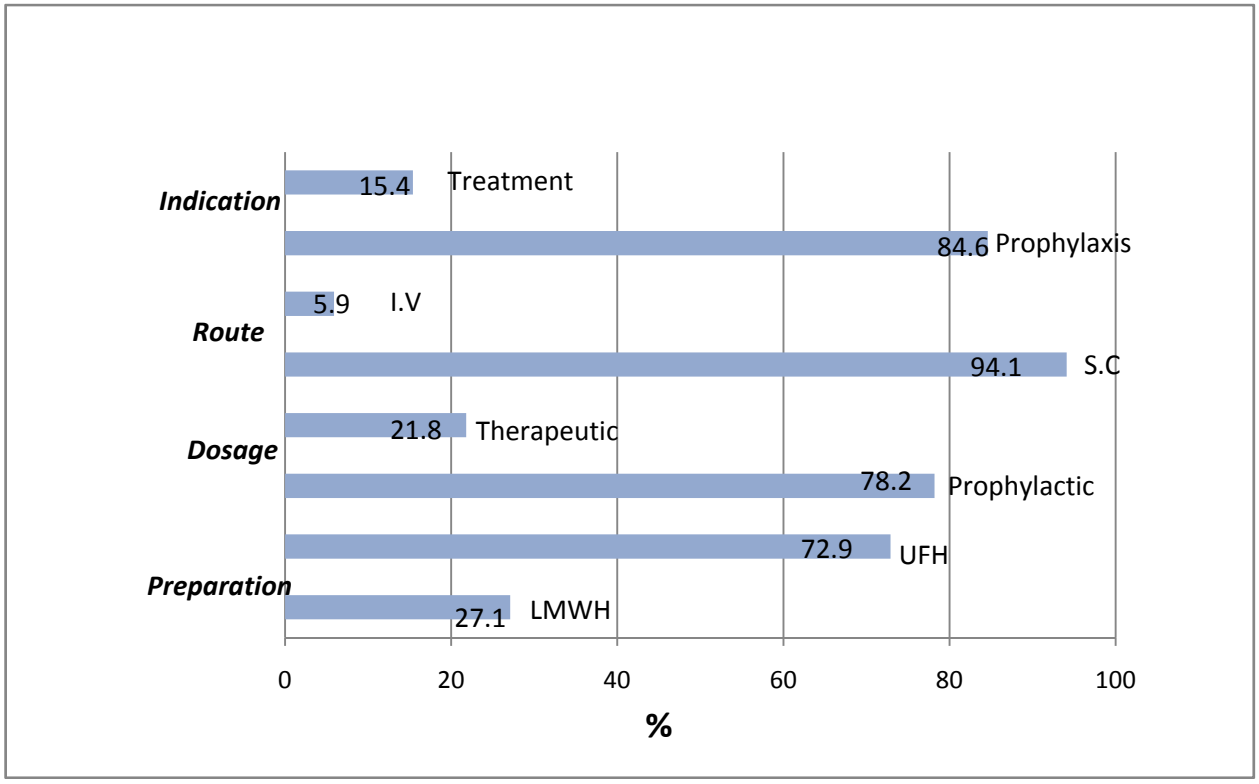
Table 2: Indications for heparin therapy in various patient groups, n = 188.

Indication	Number of patients (n)
Treatment for	
DVT (medical and obstetric)	26
P.E	2
Cardioembolic stroke	1
Medical prophylaxis	
Stroke	31
CCF	25
D.M and hypertension	19
Arrhythmias	5
Miscellaneous	16
Orthopedic prophylaxis	
ORIF (femur and tibia)	25
Hip replacement surgery	3
Spine fracture	11
Cardiac surgery prophylaxis	
Valve replacement	17
Coronary artery bypass graft	4
Obstetric prophylaxis	
Preeclampsia	3
Valvular diseases	2
Post valve replacement	1
TOTAL	***188***

Patient distribution by Heparin indication, preparation, dosage and route.

Of the 188 study patients, majority 137 (72.9%) were on UFH preparation and the rest 51 (27.1%) were on LMWH. The predominant route of administration of heparin therapy was subcutaneous (s.c.) 177 (94.1%) and rarely intravenous (i.v.) 11 (5.9%); the indication for heparin therapy was mainly prophylactic 159 (84.6%) versus treatment 29 (15.4%). Heparin dosage – majority, 147 (78.2%) were on prophylactic dosage (< 10,000U/day UFH and < 80mg/day LMWH) and the rest 41 (21.8%) were on therapeutic dosage > 10,000U/day of UFH and > 80mg/day of LMWH (Figure 4).

Figure 4: Patient distribution by Heparin indication, preparation, dosage and route (n =188)



***** at KNH treatment sheets UFH is referred to as heparin sodium and LMWH as enoxaparin.

Summary of platelet count profile

Platelet count was done for all study patients (188) from day 0, day 3 and day 7. Platelet count for the 10^{th} day was done in 141 patients only.

Non immune thrombocytopenia - (platelet count < 150 X 10^9/ L or drop by 25- 40% from baseline, occurring at 1-3 day of heparin therapy) was noted in 14 (7.4%) of the study patients. The mean of percentage platelet count drop was 32.8% (Table 4).

Heparin induced thrombocytopenia (HIT) - (platelet count less than 150 x10^9/ L or platelet count drop of > 50% from baseline occurring from 5^{th} day of heparin therapy and a positive HIA) was noted in 5 (2.7%) of the study patients. The mean of percentage platelet count drop was 55.7% (Table 2).

Table 3: Types of thrombocytopenia in relation to % platelet count drop.

	n (%)	Range of % platelet count drop.	Mean of % platelet count drop.
Nonimmune thrombocytopenia	14 (7.4)	26.1 – 39.6	32.8
HIT	5 (2.7)	50.9 – 61.2	55.7

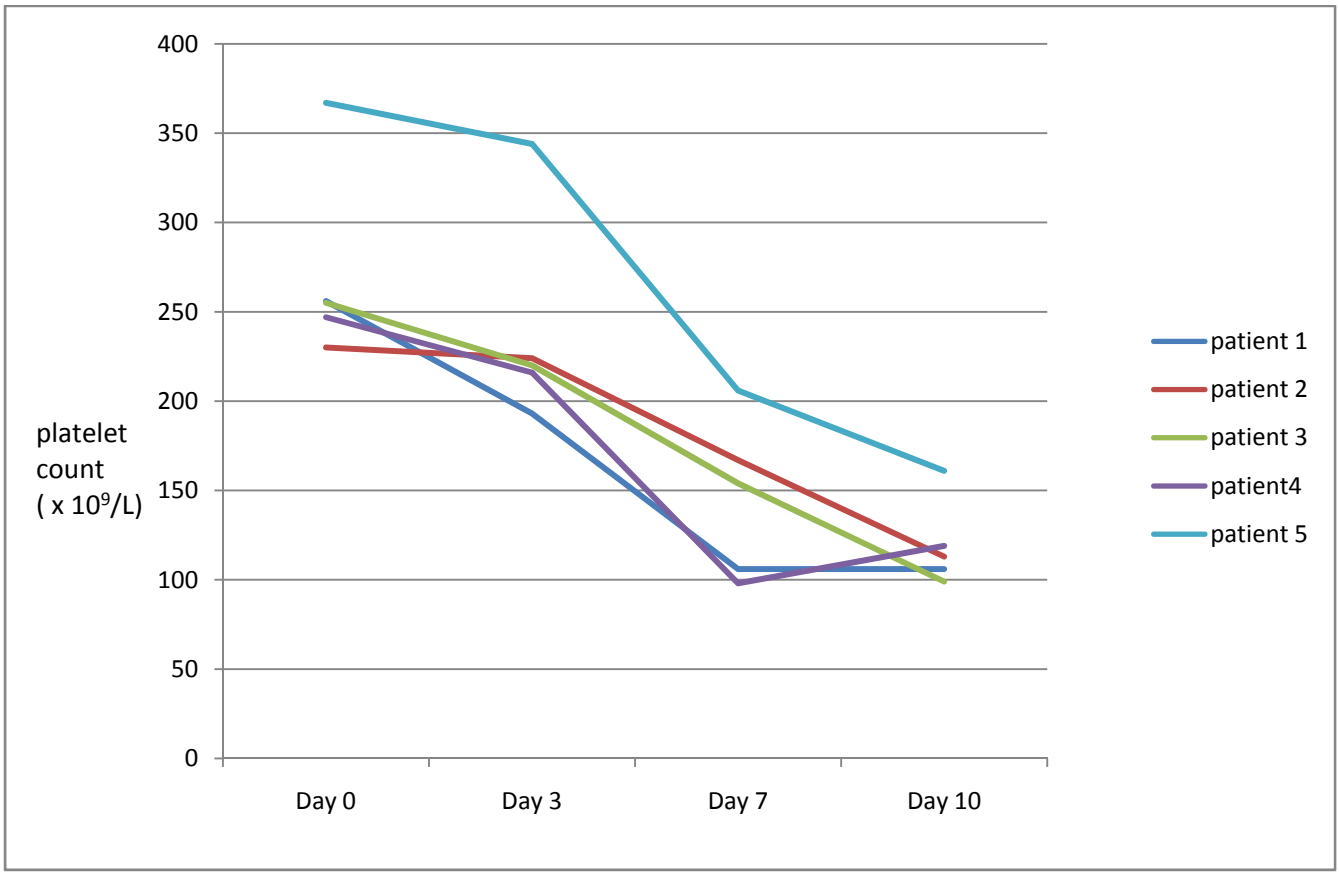
Non immune thrombocytopenia – platelet count less than 150 x10^9/ L or platelet count drop of 25 – 40% from the baseline, occurring at day 1 – 3 of heparin therapy.[46]

HIT – platelet count less than 150 x 10^9/ L or platelet count drop of more than 50% from the baseline, occurring from day 5 of heparin therapy and a positive HIA[47]

Full profile of platelet count in HIT patients

Of the 188 study patients 5 (2.7%) developed Heparin induced thrombocytopenia (HIT). They were all female from the medical ward. Three (3) of them were on heparin therapy for treatment of DVT (2) and cardioembolic stroke (1), while the rest were on prophylaxis for cerebral vascular accident (CVA) and Congestive cardiac failure (CCF).(Figure 5)

Figure 5: Full profile of platelet count in HIT patients, (n = 5)



HIT – platelet count less than 150 x 109/ L or platelet count drop of more than 50% from baseline, occurring from day 5 of heparin therapy and a positive HIA.[47]

Clinical information and HIA assay results of HIT patients

Of the 188 study patients, 5 (2.7%) developed heparin induced thrombocytopenia (HIT). The HIA assay results were positive for the five patients (ELISA O.D > 0.5). Three (3) patients were on therapeutic dosages while two (2) were on prophylactic dosages of heparin. All the five (5) patients were on UFH preparation and the route of administration was subcutaneous (s.c). (table 4).

Table 4: Clinical information and HIA assay results of HIT patients, n = 5

Age Yr.	**Sex**	**diagnosis**	**Indication**	**Type of heparin**	**Dose U/ day**	**Route**	**HIA assay results (O.D at A450)**
39	F	DVT	Treatment	UFH	17500 b.d	s.c	1.962
19	F	Cardioembolic stroke	Treatment	UFH	17500 b.d	s.c	1.394
26	F	CCF	Prophylaxis	UFH	5000 o.d	s.c	1.307
61	F	CVA	Prophylaxis	UFH	5000 o.d	s.c	0.940
45	F	DVT	Treatment	UFH	10000 t.d.s	s.c	1.422

HIA assay results (ELISA O.D at A450) : Negative ≤ 0.5 and Positive > 0.5 (see appendix 3).

Full profile of platelet count in non immune thrombocytopenia patients

Of the 188 study patients, 14 (7.4%) developed non immune thrombocytopenia, which is a platelet count decrease below 150 x 10^9/ L or drop by 25 – 40% from baseline occurring at day 1 – 3 of heparin therapy. This is an asymptomatic, benign condition of no clinical significance and does not require intervention. (Table 5).

Table 5: Full profile of platelet count and patient characteristics in non immune thrombocytopenia patients, (n =14)

	Age	Sex	Diagnosis	Platelet	Count	(x 10^9/L)	
	Yr.			Day 0	Day 3	Day 7	Day 10
1.	21	F	DVT	305	286	332	351
2.	60	M	ORIF left Femur	415	230	343	369
3.	67	M	ORIF left Femur	553	300	426	548
4.	36	F	DVT	268	173	213	-
5.	80	F	Rheumatoid arthritis	167	108	140	156
6.	56	M	CVA	529	311	392	488
7.	31	F	Demyelinating disease	217	150	255	262
8.	32	M	Transverse myelitis	418	260	381	407
9.	30	F	DVT	330	178	290	-
10.	27	F	DVT	371	213	322	389
11.	48	F	CCF	224	148	251	243
12.	75	F	Arthritis	447	305	407	-
13.	60	M	Peripheral neuropathy	392	194	255	264
14.	26	F	D. M	304	156	224	270

Clinical information and HIA assay results of non immune thrombocytopenia patients

Of the 188 study patients, 14 (7.4%) developed non immune thrombocytopenia. The HIA assay results were negative in all the fourteen patients (ELISA O.D < 0.5). Four (4) were on therapeutic doses of heparin for treatment of DVT. Two (2) were on subcutaneous prophylactic doses of LMWH for orthopedic surgery- related prophylaxis. The rest (8) were on subcutaneous prophylactic doses of UFH for medical reasons. (table 6).

Table 6: Clinical information and HIA assay results in non immune thrombocytopenia patients, n = 14

Age Yr.	Sex	Diagnosis	Indication	Type of heparin	Dose U/day or mg/day	Route	HIA assay results (O.D at A450)
21	F	DVT	Treatment	UFH	15000 b.d	s.c	0.102
60	M	ORIF left femur	Prophylaxis	LMWH	40mg o.d	s.c	0.168
67	M	ORIF left femur	Prophylaxis	LMWH	40mg o.d	s.c	0.254
36	F	DVT	Treatment	UFH	17500 b.d	s.c	0.162
80	F	Rheumatoid arthritis	prophylaxis	UFH	5000 o.d	s.c	0.133
56	M	CVA	Prophylaxis	UFH	5000 o.d	s.c	0.137
31	F	Demyelinating disease	Prophylaxis	UFH	5000 o.d	s.c	0.275
32	M	Transverse myelitis	Prophylaxis	UFH	5000 o.d	s.c	0.156
30	F	DVT	Treatment	UFH	7500 t.d.s	s.c	0.146
27	F	DVT in pregnancy	Treatment	UFH	10000 t.d.s	i.v	0.208
48	F	CCF	Prophylaxis	UFH	5000 o.d	s.c	0.185
75	F	arthritis	Prophylaxis	UFH	5000 o.d	s.c	0.250
60	M	Peripheral neuropathy	Prophylaxis	UFH	5000 o.d	s.c	0.130
26	F	D.M	Prophylaxis	UFH	5000 o.d	s.c	0.166

HIA assay results (ELISA O.D at A450): Negative $\leq$ 0.5 and Positive > 0.5 (see appendix 3).

Correlation between Heparin induced thrombocytopenia (HIT) and demographic variables

Of the 188 study patients, 5 (2.7%) developed heparin induced thrombocytopenia (HIT). All the 5 (100%) patients were female and none (0%) were male. Of the five (5) patients with HIT, 3 (60%) were aged 40 years and below while 2 (40%) were aged above 40 years.

There was no statistical significant difference between HIT status and sex or age of the patients (Table 7).

Table 7: Correlation between HIT and demographic variables (n = 188)

Variable	**HIT Status**		**O.R. (C.I)**	**P-value**
	Yes n (%)	**No n (%)**		
Gender				
Male	0 (0)	79 (43.2)		
Female	5 (100)	104 (56.8)	_	0.054
Age				
≤ 40 years	3 (60)	96 (52.5)		
> 40 years	2 (40)	87 (47.5)	1.4 (0.22-8.33)	0.739

Correlation between HIT and heparin preparation, dosage and route.

Of the 5 study patients who developed HIT, all (100%) were on UFH preparation. The route of administration of heparin therapy for all 5 (100%) patients was subcutaneous (s.c). 3 (60%) of patients with HIT were on a therapeutic dosage of heparin (> 10,000U/day UFH and > 80mg/day LMWH) while 2 (40%) were on prophylactic dosage of heparin (≤ 10,000U/day UFH and ≤ 80mg/day LMWH).

Heparin preparation, dosage and route had no significant influence on the development of HIT (Table 8).

Table 8: Correlation between HIT and heparin preparation, dosage and route. (n = 188)

Characteristic	HIT Status		O.R (C.I)	p-value
	Yes n (%)	No n (%)		
Preparation				
* LMWH	0 (0)	51 (27.9)		
*UFH	5 (100)	132 (72.1)	_	0.167
Dosage				
Prophylactic	2 (40)	145 (79.2)		
Therapeutic	3 (60)	38 (20.8)	0.2 (0.03-1.08)	0.07
Route				
I.V.	0 (0)	11 (6)		
S.C	5 (100)	172 (94)	_	0.572

*****at KNH treatment sheets UFH is referred to as heparin sodium and LMWH as enoxaparin.

HIA assay results

Heparin induced antibody (HIA) detection was carried out in all the study patients in either day seven (7) or day ten (10) since heparin therapy initiation. Of the 188 study patients, 32 (17%) were HIA positive while 156 (83%) were HIA negative.

Of the 32 HIA positive study patients only five (5) developed immune thrombocytopenia (HIT).

Of the 156 HIA negative study patients, 14 of them developed non-immune thrombocytopenia which is a benign disorder and does not require treatment. None of the HIA negative study patients developed HIT.

Most of the HIA positive patients were on UFH (25 patients) while 7 were on LMWH; 21 were on prophylactic heparin dosage while 11 were on therapeutic dosage; most of them the route of heparin administration was subcutaneous (31 study patients) and only one received intravenous heparin, (Table 9).

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Table 9: Clinical information and HIA assay results of HIA Positive study patients (n=32)

Age (yr)	Sex	Diagnosis	Indication	Type of heparin	Dose/ day	Route	Optic density
39	F	DVT	Treatment	UFH	17,500U b.d	s.c	1.962
19	F	Cardio-embolic stroke	Treatment	UFH	17,500U b.d	s.c	1.394
26	F	CCF	Prophylaxis	UFH	5000U o.d	s.c	1.307
61	F	CVA	Prophylaxis	UFH	5000U o.d	s.c	0.940
45	F	DVT	Treatment	UFH	10,000U t.d.s	s.c	1.422
19	M	Spine fracture	Prophylaxis	LMWH	40mg o.d	s.c	0.634
39	F	CCF	Prophylaxis	UFH	5000U o.d	s.c	0.517
51	M	ORIF right femur	Prophylaxis	LMWH	40mg o.d	s.c	0.522
37	M	DVT	Treatment	LMWH	80mg b.d	s.c	0.751
60	F	Spine fracture	Prophylaxis	LMWH	40mg o.d	s.c	0.597
28	F	DVT	Treatment	UFH	17,500U b.d	s.c	0.825
40	F	Arrhythmias	Prophylaxis	UFH	5000U o.d	s.c	0.711
43	M	CVA	Treatment	UFH	17,500U b.d	s.c	0.833
59	M	D.M & Hypertension	Prophylaxis	UFH	5000U o.d	s.c	0.510
34	F	P.E	Treatment	UFH	10,000U t.d.s	s.c	0.727
70	F	D.M	Prophylaxis	UFH	5000U o.d	s.c	0.521
36	F	Peripheral neuropathy	Prophylaxis	LMWH	40mg o.d	s.c	0.540
26	F	Acute Guillain-Barre	Prophylaxis	UFH	5000U o.d	s.c	0.569
54	F	CVA	Prophylaxis	UFH	5000U o.d	s.c	0.516

Table 9 (continuing): Clinical information and HIA assay results of HIA positive study patients (n=32)

Age (yr)	sex	Diagnosis	Indication	Type of heparin	Dose / day	Route	Optic density
45	F	CCF	Prophylaxis	UFH	5000U o.d	s.c	0.622
20	F	CCF	Prophylaxis	UFH	5000U o.d	s.c	0.867
50	F	Valve replacement	Prophylaxis	UFH	5000U t.d.s	s.c	0.589
29	F	DVT in pregnancy	Treatment	UFH	7,500U t.d.s	i.v	0.835
32	F	ORIF left femur	Prophylaxis	LMWH	40mg o.d	s.c	0.539
34	M	D.M	Prophylaxis	UFH	5000U o.d	s.c	0.507
71	M	CVA	Prophylaxis	UFH	5000U o.d	s.c	0.590
65	M	D.M & Hypertension	Prophylaxis	UFH	5000U o.d	s.c	0.539
32	F	Arrhythmias	Prophylaxis	UFH	5000U o.d	s.c	0.641
27	F	D.M	Prophylaxis	UFH	5000U o.d	s.c	0.711
45	F	DVT	Treatment	UFH	10,000U t.d.s	s.c	0.651
65	M	Hip replacement surgery	Prophylaxis	LMWH	40mg o.d	s.c	0.511
38	M	DVT	Treatment	UFH	17,500U b.d	s.c	0.613

Prevalence of HIA according to heparin preparation and various wards.

The majority of study patients 137(72.9%) were on UFH preparation and the rest 51 (27.1%) on LMWH preparation. HIA positivity was observed in 25 (18.2%) of those on UFH and in 7 (13.7%) of those on LMWH.

The medical ward had 111 patients, of whom 24 (21.6%) were HIA positive, orthopedic surgery ward 5 (12.8%) of them out of 39 patients were HIA positive, cardiothoracic surgery ward 2 (9.5%) out of 21 were HIA positive and 1 (5.9%) out of 17 patients in obstetric ward were HIA positive (Table 10)

Table 10: Prevalence of HIA according to heparin preparation and various wards, (n = 188).

Characteristic	HIA Positive (n)	Total number of patients (n)	Prevalence (%)
Heparin preparation			
UFH	25	137	18.2
LMWH	7	51	13.7
Total	*32*	*188*	
Wards			
Medical	24	111	21.6
Orthopedic surgery	5	39	12.8
Cardiothoracic surgery	2	21	9.5
Obstetric	1	17	5.9
Total	*32*	*188*	

Correlation between HIA positivity and the various demographic variables

The majority 22 (68.75%) of the patients who were HIA positive were female while the rest 10 (31.25%) were male. The majority of them 17 (53.1%) were 40 years and below while the rest 15 (46.9%) were over 40 years (Table 11).

There was no statistical significant difference in the HIA positivity between male and female (p-value 0.175), as well as between those aged below or above 40 years (p-value 0.954).

Table 11: Correlation between HIA positivity and various demographic variables

Variable	HIA Status		O.R. (C.I)	P-value
	Positive n (%)	Negative n (%)		
Gender				
Male	10 (31.25)	69 (44.2)		
Female	22 (68.75)	87 (55.8)	0.6 (0.26-1.29)	0.175
Age				
< 40 years	17 (53.1)	82 (52.6)		
> 40 years	15 (46.9)	74 (47.4)	1.02 (0.48-2.19)	0.954

Correlation between HIA positivity and various patient categories

There were 32 (17%) study patients who were HIA positive. The majority of these were from medical wards 24 (75%), orthopaedic surgical wards has 5 (15.6%), cardiothoracic surgical ward has 2 (6.3%) and only 1 (3.1%) patient was from Obstetric ward (Table 12).

There was no statistical significant relationship between the HIA positivity and any of the four wards (p-value 0.214)

Table 12: Correlation between HIA positivity and various patient categories, (n = 188)

Unit	**HIA**		**Status**		**p-value**
	Positive	n (%)	Negative	n (%)	
Cardiothoracic surgery	2 (6.3)		19 (12.2)		
Orthopedic surgery	5 (15.6)		34 (21.8)		
					0.214
Medical	24 (75)		87 (55.8)		
Obstetric	1 (3.1)		16 (10.2)		

Correlation between HIA positivity and heparin indication, preparation, dosage and route.

The majority 25 (78.1%) of the HIA positive patients were given heparin therapy for prophylactic purposes and only 7 (21.9%) were prescribed heparin for treatment purposes. Likewise the majority 25 (78.1) were on unfractionated heparin (UFH) and 7 (21.9%) were on low molecular weight heparin preparation (LMWH). Most of HIA positive study patients 21 (65.6%) were on prophylactic heparin dosage ($\leq$ 10,000U/day UFH and $\leq$ 80mg/day LMWH), the route of heparin therapy administration was mainly subcutaneous (s.c.) 31 (96.9%).

There was no statistically significant relationship between HIA positivity and heparin indication, preparation, dosage and route (Table 13).

Table 13: Correlation between HIA positivity and heparin indication, preparation, dosage and route. (n = 188)

Characteristic	HIA Status		O.R (C.I)	P-value
	Positive n(%)	Negative n(%)		
Indication				
Prophylaxis	25 (78.1)	134 (85.9)		
Treatment	7 (21.9)	22 (14.1)	0.6 (0.23-1.52)	0.267
Preparation				
* LMWH	7 (21.9)	44 (28.2)		
*UFH	25 (78.1)	112 (71.8)	0.7 (0.29-1.77)	0.463
Dosage				
Prophylactic	21 (65.6)	126 (80.8)		
Therapeutic	11 (34.4)	30 (19.2)	0.5 (0.20-1.04)	0.059
Route				
I.V	1 (3.1)	10 (6.4)		
S.C	31 (96.9)	146 (93.6)	0.5 (0.06-3.82)	0.471

*at KNH treatment sheets UFH is referred to as heparin sodium and LMWH as enoxaparin.

DISCUSSION

Heparin induced thrombocytopenia (HIT) and its manifestations, skin necrosis and anaphylactic reactions are well recognized complications of heparin after bleeding complications [43]. This study was able to screen for these complications and bring out clinically relevant information which can initiate further studies. Of the three complications HIT was found at a low prevalence among the study patients. Skin necrosis and anaphylactic reactions were not observed in the study patients.

Platelet count monitoring was done for all study patients (188) except on day 10 where 47 patients were missing due to clinical discharges. Non immune thrombocytopenia which is mild, asymptomatic and of little clinical consequence was noted in 7.4% of the study patients. This is slightly lower prevalence compared to other studies where the prevalence was shown to range between 10% and 25% [3, 46]. This appears so because in previous studies the monitoring of platelet count was done at least every other day from initiation of heparin therapy, while in this study it was done after 2 days, therefore there is a possibility that some cases may have been missed in day 1 and 2 [3, 46].

The non immune thrombocytopenia patients developed a platelet count drop of 25 – 40% from baseline which occurred at day 1 – 3 of heparin therapy. This platelet count drop could also be due to immune reasons as suggested by other authors, but with the use of pretest probability scoring system of HIT (the 4Ts) all the patients had a low score of either 1/8 or 2/8 which indicates a low probability towards immune thrombocytopenia (see appendix 4)[48].

The overall prevalence of HIT was 2.7% (3.65% with UFH and 0% with LMWH). This seems to compare well with previous studies where the prevalence has been reported to range between 1% and 5%, though the prevalence of HIT varies greatly depending on the type of heparin preparation, the duration of therapy and most importantly the patient population (whether cardiothoracic surgery, orthopaedic surgery, medical or obstetric) [9, 10]. All the patients with HIT were positive for the HIA assay. Two (40%) of the patients with HIT had ELISA O.D of > 1.4 which indicates a high probability for a positive SRA, the Gold standard for HIA detection. (see appendix 5)[49].

In this study all the patients with HIT were on UFH preparation. These results are in agreement with previous studies that demonstrated patients on UFH had higher prevalence of HIT compared to those on LMWH (0.53% with UFH and 0% with LMWH -Lindhoff-Last et al, 2002;

2.7% with UFH and 0% with LMWH – Warkentin et al, 1995) [9, 10]. In this study there was no statistical significant difference between HIT status and preparation of heparin therapy (p-value 0.167).

All the patients with HIT were medical ward patients. This differs with previous studies in which the highest frequency of patients with HIT were orthopaedic patients, with medical and obstetric patients having the lowest frequency [8,14,50]. The reason for this contradiction could be due to the fact that almost all patients from orthopaedic surgery unit were on LMWH preparation which is known to have a lesser risk for HIT compared to UFH [9, 10.] In patients who underwent cardiac surgery most of them were on heparin for a short duration (2 -3 days) and switched on to warfarin. This might have contributed to the low prevalence of HIT in surgical patients as it is well known that the risk of HIT increases with duration of heparin therapy[10, 51]. Also there are no extensive studies which have been done on medical patients; most studies have concentrated on cardiac surgery and orthopaedic surgery patients [10]. HIT was not detected in the other patient categories (cardiac surgery, orthopaedic and obstetric).

The slightly higher prevalence of HIT in medical patients which is in contradiction with previous studies where the prevalence was low (0.8%),[50] could also be possibly due to the fact that in other studies more strict criteria and more laboratory tests were used to exclude other causes of thrombocytopenia.[52] Also in other studies done in neurologic patients the prevalence of HIT appeared to be higher (2.5%) and this study had several neurologic patients one of which developed HIT.[11] Again as pointed out earlier there are no extensive studies which have been done on medical patients hence the prevalence could be underestimated.[10]

This study could not correlate the HIT status and duration of therapy. This could be attributed to the design of this study where follow up was done for ten (10) days only which falls within a short duration (in other studies a longer duration was used and defined as heparin therapy for more than twenty eight (28) days) [10]. However in other studies it has been shown that the risk of HIT increases with the duration of heparin therapy [10,51].

There was no statistically significant difference in the HIT status between sexes (p-value 0.054) as well as between those aged < 40 years and those aged > 40 years (p-value 0.739). This is in comparison with previous studies where age and sex have been shown to have no influence in the development of HIT.[10] However in this study all the patients with HIT were females and the p-value of 0.054 shows a statistical trend to significance which would become more obvious in a larger study population.

This study did not demonstrate any statistically significant difference in the HIT status between intravenous route of administration and subcutaneous route (p-value 0.572); prophylactic dosage (≤ 10,000U/day UFH and ≤ 80mg/day LMWH) and therapeutic dosage of heparin therapy (> 10,000U/day UFH and > 80mg/day LMWH), (p-value 0.07). Some of the studies reported in literature have associated both high therapeutic doses and intravenous route of heparin administration with the development of HIT, however in other studies involving medical patients only, HIT developed exclusively in those on subcutaneous prophylactic doses of heparin.[52, 53, 54] In this study the statistical trend (p-value 0.07) suggests a relationship between HIT and high therapeutic doses despite the fact that few patients were on therapeutic doses. Perhaps this would become more significant in a larger population.

Skin necrosis was not observed in this study. None of the study patients was noted to have either erythematous plaques or frank skin necrosis at the site of heparin therapy administration. These results are almost in agreement with previous literature reports where the prevalence of skin necrosis has been noted to be very low, developing in 10-20% of patients who are HIA positive [43]. Which means for this study with thirty two (32) HIA positive patients, the expected number of patients having skin necrosis would have been approximately three to six (3 – 6). The low prevalence in this study could be due to the fact that about 25% of the study patients were followed up to the 7th day only, while skin necrosis is known to occur from 7th up to 10th day from initiation of heparin therapy. [38,39,43.] Secondly skin biopsies were not performed in this study to confirm suspicious lesions and thirdly the study design used in previous studies was a nested cohort study comparing HIA positive patients and HIA negative patients, who were followed up for unusual clinical sequelae, all were from orthopaedic surgery. This study was a cross sectional study and had involved four different patient populations [43]. Perhaps there could be an association between skin necrosis and the patient population.

Heparin induced anaphylactic reactions were not observed in this study. The signs and symptoms of anaphylactic reactions like urticaria, angioedema, dyspnoea, bronchospasm and anaphylactic shock, which usually develop minutes after heparin therapy administration, were not observed or reported in the study patients. These results concur with previous literature reports that showed anaphylactic reactions are very rare and documented only as case reports[42, 44, 45]. This is supported again by a nested cohort study in 2000 where twenty (20) HIA positive patients were followed up for unusual clinical sequelae, and only one patient developed an anaphylactic reaction [8].

The ELISA for heparin induced antibody detection was performed in all the study patients (188) on either day 7 or 10 of heparin therapy initiation. The overall prevalence of heparin induced antibodies (HIA) among study patients on heparin therapy was 17%. This means 17% of study patients on heparin therapy were at risk of developing the three heparin associated complications (HIT, skin necrosis and anaphylactic reactions). From previous studies, most of which were carried out in North America and Europe, the overall frequency of both HIA and HIT are difficult to precisely define because the risk is associated with the type of heparin, the duration of heparin therapy, the study design and most importantly the patient population[10].

Due to various reasons validation of the ELISA for heparin antibody detection and setting up of the local cut off values was not done, instead the cut off values provided by the kit's manufacturer were used (OD of more than 0.5 was considered positive). As it was noted in the results the study patients who developed immune thrombocytopenia had an O.D values greater than 0.9.

There are many guidelines on the interpretation of the HIT antibody test and most recommend the use of high dose heparin (100U/ ml). Inhibition of a positive result (more than 50% reduction of the O.D) by high concentration of heparin is characteristic of HIT antibodies[55]. This step was not performed in this study. If performed it would have further confirmed the HIT antibody test results and probably would have an impact on the prevalence of this antibodies.

Most of the previous studies were patient type specific, involving only one patient population as in cardiac surgery patients only or medical patients only. Rarely studies involving more than one patient population have been conducted, [8,10,27] while this study involved four different patient populations.

The prevalence of HIA was highest in medical patients 21.60%, followed by orthopaedic patients 12.80%, then cardiac surgery patients 9.80% and lastly obstetric patients 5.90%, though this difference was not statistically significant. These results are not in agreement with previous studies where the highest prevalence of antibody formation was seen in the cardiac surgery patients 50% and medical patients having a much lower prevalence 9.20% [8, 50]. This could be explained by the fact that most cardiac surgery and orthopaedic surgery patients were given the therapy for a short duration, usually for 2-3 days, while it is known that antibody formation increases with duration of heparin therapy [10].

The prevalence of heparin induced antibodies (HIA) according to heparin preparation/ type among study patients was 18.2% with UFH and 13.7% with LMWH. These results concur with previous studies where the prevalence of HIA was higher with UFH than LMWH (Warkentin et al, 1995 – 7.8% with UFH and 2.2% with LMWH; Pouplard et al, 1999 – 29% with UFH and 21.60% with LMWH) [9, 56]. However there was no statistical significance in the difference of HIA positivity between the two preparations.

There was no statistical significant difference in the prevalence of heparin induced antibodies between sexes (p-value 0.175) as well as between those aged below or above 40 years (p-value 0.954). Age and sex have not been shown to influence antibody formation in previous studies.[10]

This study did not demonstrate any statistically significant difference in the HIA positivity between - the two indication for therapy, prophylactic versus treatment (p-value 0.267); intravenous route and subcutaneous route of heparin therapy administration (p-value 0.471); prophylactic dosage ($\leq$ 10,000U/day UFH and $\leq$ 80mg/day LMWH) and therapeutic dosage (> 10,000U/day UFH and > 80mg/day LMWH), (p-value 0.059). The p-value of 0.059 shows a statistical trend suggesting significant association between high therapeutic doses and development of heparin induced antibodies which is also evident in several other studies.[11, 57]

The majority of the study patients on heparin therapy were female. This could be explained by the fact that there are more females than males in the total adult patient population at KNH (unpublished data – KNH medical records, inpatient statistics according to gender, January to December 2009).

The highest proportion of the study patients were in the age bracket 30-34 years while the least proportion was in the age bracket 55-59 years. Factual explanation for this particular entity was not found.

Most of the study patients were from the medical wards. Medical inpatient department in this study was the largest comprising of eight (8) wards. Cardiothoracic ward, which had fewer patients, it is comprised of only one ward, with approximately 1 to 2 cardiac surgeries conducted in a week. The least number of patients were from obstetric though composed of three (3) wards, most of them were on treatment for DVT in pregnancy, with approximately 2 to 3 patients admitted in a week, but most of them were discharged before day 7 since heparin therapy initiation.

Most of the patients were on UFH preparation as it is easily available, cheaper and supplied by the hospital pharmacy[14]. The route of administration of heparin therapy in most wards was mainly subcutaneous which is preferred by the care staff for ease and convenience. The indication for heparin therapy in the majority of patients was prophylactic hence most of them were on prophylactic doses of heparin ($\leq$ 10,000U/day UFH and $\leq$ 80mg/day LMWH).

There are no documented studies in literature in the African population for these uncommon but serious side effects of heparin therapy, even though the use and indication of this drug continues to rise due to increased risks of thromboembolic disorders[3]. This is a baseline study in a setting where UFH is still used when most practices elsewhere have shifted to the lesser risk associated LMWH. The findings of this study may be used to design further studies where the heparin complications can be better studied and proper characterization of the risk factors done which may as well be different in our African population.

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

1. Heparin induced thrombocytopenia (HIT) syndrome is a rare complication of heparin at KNH, with a prevalence of 2.7%. Its unusual manifestations skin necrosis and anaphylactic reactions were not seen in this study.

2. Heparin induced antibody (HIA) which is associated with the risk of developing HIT, skin necrosis and anaphylactic reactions was found at a prevalence of 17%. However as the validation of the HIT antibody test and some of the steps in the interpretation of the HIT antibody test (inhibition of a positive result by high concentration of heparin) were not performed, there is a probability that the prevalence of this antibody could be much lower.

3. With the low prevalence of HIT and the limitations of this study, routine screening of the HIT antibody at KNH may not be useful until further studies are carried out.

4. It can be concluded from this baseline study that heparin is a fairly well tolerated drug among patients at the KNH.

Recommendations

1. Although HIT, skin necrosis and anaphylactic reactions are rare and with a relatively low prevalence of HIT, it is still advised to perform a close clinical and laboratory surveillance (e.g. platelet count) in all patients on prophylactic and therapeutic doses of UFH.

2. Further studies are needed in KNH, particularly based on patient population-specific for a more accurate determination of the prevalence of HIT and other complications, as patient population has been shown to have a strong association with HIT in other studies elsewhere.

3. More advanced studies on HIT are recommended which will involve more extensive laboratory investigations to exclude other causes of thrombocytopenia, measures to

confirm thrombosis (Doppler ultrasonography, venography) and probably longer duration of follow up to enable proper approximation of the prevalence of HIT and skin necrosis.

STUDY LIMITATIONS

1. Comprehensive exclusion of other causes of thrombocytopenia before and during the study was not exhaustively done in this baseline study. Only a full blood picture was done and the rest (if performed) were obtained from the patient's medical records.
2. Estimation of platelet count could have been affected by pre-, analytical and post analytical factors that were beyond the control of this study.
3. Patient follow up in this study was only up to 10 days yet complications such as HIT and skin necrosis are known to occur beyond 10 days of heparin therapy.
4. Some of the anaphylactic reactions could have been missed as this study partly relied on patients' medical records for any documented signs and symptoms of anaphylactic reactions, which might have occurred but was not documented.
5. The ELISA method for detection of HIA has a moderate specificity, and gold standard for confirmation of these antibodies (SRA) was not done. Indeed the SRA is usually done at a reference laboratory.
6. Measures to confirm or exclude thrombosis such as Doppler ultrasonography and venography were not performed in this study.

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APPENDICES

Appendix 1: Patient information and consent form.

Information sheet:

Heparin the commonly used anticoagulant in our setting has a number of serious side effects besides bleeding. This includes thrombocytopenia with thrombosis, skin necrosis, and anaphylactic reactions.

This study is aimed at determining the presence of heparin induced antibodies which has been associated with development of these complications, as well as presence of thrombocytopenia, skin necrosis and anaphylactic reactions in patients on heparin therapy.

First an interview and physical examination will be performed to allow proper selection of patients. The selected patients will be required to sign the consent form, and thereafter filling of a questionnaire regarding patient's characteristics. Then venous blood (2ml) from the forearm will be collected before heparin therapy initiation, then on 3^{rd}, 7^{th} and 10^{th} day (about four samples in total) for platelet count monitoring. For HIT antibody detection 4mls of venous blood will be collected at the same site on the 10^{th} day. Physical examination will be performed on similar days for evidence of skin necrosis and anaphylactic reactions.

Risks and benefits of this study – risks anticipated are minor and include pain at the site of injection and sometimes hematoma formation which will subside within days. The benefit includes detection of any abnormalities (HIA & thrombocytopenia, skin necrosis and anaphylactic reactions) that may be present. The results obtained in this study will be communicated to the attending doctor immediately for management.

Ethical issues – This study has been approved by the ethical and research committee of this hospital. The results of this test will be sent back to you through your doctor.

You will not be charged for the laboratory tests involved in this study. The blood sample as well as results from this study will only be used for the above purposes and no other.

Confidentiality – your identity will be kept strictly confidential throughout the study as well as during the publication of the study findings. Your decision to participate or not to participate in this study will not affect the quality of your care.

Kindly fill the consent form below:

Consent form

I Mr. /Mrs. _____ Agree to enroll myself into this study being fully aware of its purpose as explained to me by_____, and consent to the investigations.

Signature:

a) Participant/ relative _____ Date_____ .

b) Witness _____ Date_____ .

During this study, if you have any concerns contact me Dr. MSAFIRI M.L (Principal investigator) on telephone number 0723023836 or my supervisors Dr. RAJAB J.A. (telephone number 0722707421) and Dr. DAVE P. (telephone number 0733594299).

If you have further questions about the study you can contact Prof. BHATT K, Chairperson of Ethics Committee on telephone number 02726300 (extension 44102).

Appendix 2: Data collection instrument (Study Questionnaire).

Study title: Heparin associated complications in adult patients at KNH.

1. Study number | | | |

2. Code | | | |

Part A: Information from patient's files

3. Patient's name _____ .

4. IP/NO. | | | | | | | |

5. Age | | | (years).

6. Address (district) _____ .

7. Sex 1) Male.

2) Female.

8. Ward/ unit: 1) Orthopedic surgical ward.

2) Cardiovascular surgical ward.

3) Medical ward.

4) Obstetric ward.

5) Other _____.

9. Diagnosis _____.

10. History of previous exposure to heparin

1) Yes 2) No

10. Type of heparin preparation prescribed

1) LMWH 2) UFH

11. Route of administration of heparin therapy

1) I.V 2) S.C

12. Indication for heparin therapy

1) Prophylaxis.

2) Treatment.

13. Duration of heparin therapy _____ (days).

1) < 7 days. 2) > 7 days.

14. Heparin dosage: 1) <10,000U 2) > 10,000U.

Part B: Laboratory tests

15. Platelet count monitoring

Date.				
Day (since onset of heparin therapy).	**0**	**3rd**	**7th**	**10th**
Platelet count (per liter).				

16. Thrombocytopenia Type I: 1) Yes. 2) No.

17. Thrombocytopenia Type II: 1) Yes. 2) No.

18. HIT antibody status:

1) Positive.

2) Negative.

Part C: Physical examination

19. Presence of skin necrosis:

1) Erythematous (red) plaque. (Day of onset since heparin initiation _____).

2) Necrosis. (Day of onset since heparin initiation _____).

3) None.

20. Reported/ observed anaphylactic reaction:

1) Urticaria.

2) Angioedema.

3) Chest pain, breathlessness & bronchospasm.

4) Shock.

5) None.

Appendix 3

Assay procedure for Heparin induced antibody (HIA) detection

This was done by the ZYMUTEST HIA, IgG ELISA kit, which is a qualitative assay intended for the detection of heparin dependent antibodies of the IgG isotype.

The procedure was carried out following instructions as per the kit's manufacturer.

The details of the procedure and the interpretation of the test results are as per the attached kit's instructions hereunder.

CE

ZYMUTEST HIA IgG

(# RK040A)

Qualitative assay for the detection of heparin-dependent antibodies of the IgG isotype by ELISA

For in vitro diagnostic use only

HYPHEN BioMed

ZAC Neuville Université – 155, rue d'Eragny
95000 Neuville-sur-Oise – France
Tél. : 01 34 40 65 10 – Fax : 01 34 48 72 36
www.hyphen-biomed.com

Last revision: 23/10/2007

INTENDED USE:

The ZYMUTEST HIA, IgG ELISA kit, is a qualitative assay intended for the detection of heparin-dependent antibodies of the IgG isotype, in human plasma, by clinical laboratories. It is intended for in vitro diagnostic use.

ASSAY PRINCIPLE:

The diluted assayed plasma sample is introduced into one of the microwells of the coated plate, and supplemented with a platelet lysate. When present, heparin-dependent antibodies, of the IgG isotype, form complexes onto the biologically available unfractionated heparin, immobilised and saturated. Following a washing step, bound antibodies are revealed with the immunoconjugate, which is made of goat polyclonal antibodies anti-human IgG (Fcγ specific)-peroxidase (HRP) conjugate. This immunoconjugate reacts specifically with IgG isotypes. Following a new washing step, the peroxidase substrate, Tetramethylbenzidine (TMB) in presence of hydrogen peroxide (H_2O_2), is introduced and a blue colour develops. The colour turns yellow when the reaction is stopped with sulfuric acid. The colour developed is directly proportional to the amount of heparin-dependent antibodies, of the IgG isotype, present in the tested sample.

TESTED SAMPLES:

– Trisodium citrate anticoagulated human plasma.

REAGENTS:

1. **COAT: Micro ELISA plate,** containing 12 strips of 8 wells, coated with unfractionated heparin, biologically available, saturated, then stabilized; the plate is packed in an aluminium pouch hermetically sealed in presence of a desiccant.
2. **SD**: 2 vials containing 50 ml of **HIA Sample Diluent**, ready to use. Contains Sodium Azide
3. **C+**: 3 vials of **HIA IgG Positive control**, lyophilised. When restored with **1 ml** of **HIA Sample Diluent**, the ready to use positive control is obtained (already diluted 1:100). The expected reactivity is indicated on the flyer provided with the kit.
4. **C-:** 3 vials of **negative control**, lyophilised (diluted normal human plasma). When restored with **1 ml** of **HIA Sample Diluent**, the ready to use negative control is obtained (already diluted 1:100).
5. **CLy:** 3 vials of **cell lysate,** lyophilised (diluted normal human plasma). When restored with **2 ml** of **distilled water**, the ready to use solution is obtained.
6. **IC:** 3 vials of **immunoconjugate (Anti-IgG (Fcγ)-HRP immunoconjugate)**, goat antibodies specific for human IgG (Fcγ)- coupled to HRP, lyophilised. When restored with 7.5 ml of Conjugate Diluent (CD), the ready to use immunoconjugate is obtained.
7. **CD:** 1 vial of 25 ml of **conjugate diluent**, ready to use.
8. **WS**: 1 vial of 50 ml of 20 fold concentrated **Wash Solution**.
9. **TMB:** 1 vial of 25 ml peroxidase substrate: 3,3',5,5' – **Tetramethylbenzidine** containing hydrogen peroxide, ready to use.
10. **SA:** 1 vial of 6 ml of **0.45M Sulfuric Acid (Stop Solution)**, ready to use.

Note: Use only components from a same kit lot number. Do not mix components from different lots when running the assay.

REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED:

- **8-channel** or **repeating pipette** allowing dispensing 50-300 µl.
- **1-channel pipettes** at variable volumes from 0 to 20 µl, 20 to 200 µl and 200 to 1000 µl.
- **Micro ELISA plate washing equipment (and shaker)**.
- **Micro ELISA plate reader** with a wavelength set up at 450 nm.
- **Distilled water**.

REAGENTS PREPARATION, STORAGE AND STABILITY:

In their original packaging box, before use, when stored at 2-8°C, the unopened reagents are stable until the expiration date printed on the box.

1. **Micro ELISA plate:** open the plastic pouch and take off the required amount of 8 well strips for the test series. When out of the pouch, the strips must be used within 30 minutes. Unused strips can be stored at **2-8°C** for **8 weeks** in their original aluminium pouch, in presence of the desiccant, hermetically closed and protected from any moisture, and stored in the provided microplate storage bag (minigrip).
2. **HIA Sample Diluent:** It is ready to use. When open, it can be used for **8 weeks**, stored at **2-8 °C**, and provided that it remains protected from any bacterial contamination. This reagent contains sodium azide.
3.

Warning: The **HIA Sample Diluent** contains sodium azide, which may react with lead and copper plumbing to form highly explosive metal azides. Flush with large volumes of water when discarding into a sink.

4. **HIA IgG Positive Control:** restore each vial with **1 ml** HIA sample diluent in order to obtain the ready to use positive control. It corresponds to a plasma containing IgG isotype heparin-dependent antibodies, already **diluted 1:100**. Following reconstitution, the positive control is stable for **2 weeks at 2-8°C**, provided that it remains protected from any bacterial contamination, or **2 months** at **-20°C** or below.
5. **Negative control:** restore each vial with **1 ml** HIA sample diluent in order to obtain the ready to use negative control. It corresponds to a normal human plasma, already **diluted 1:100**. Following reconstitution, the negative control is stable for **2 weeks at 2-8°C**, provided that it remains protected from any bacterial contamination, or **2 months** at **-20°C** or below.
6. **CLy:** restore each vial with **2 ml** distilled water in order to obtain the ready to use reagent. Following reconstitution, the reagent is stable for **2 weeks at 2-8°C**, provided that it remains protected from any bacterial contamination, or **2 months** at **-20°C** or below.

Warning: The CLy used for the assay is extracted from fresh human platelet concentrates. The negative control is also prepared with human plasma, tested with registered methods and found negative for HIV antibodies, HBs Ag and HCV antibodies. However, no assay may warrant the total absence of infectious agents. Any product of human origin must then be handled with all the required cautions, as being potentially infectious.

6. **Anti-IgG (Fcγ)-HRP immunoconjugate**: each vial must be restored with **7.5 ml of conjugate diluent**. Let the pellet to be completely dissolved before use, and shake the vial gently in order to homogenize the content. The restored conjugate is stable for at least **24 hours** at **room temperature** or for at least **4 weeks at 2-8°C,** or **2 months** at **-20°C** or below.
7. **Conjugate diluent**: It is ready to use. When open, it can be used for **8 weeks**, stored at **2-8 °C**, and provided that it remains protected from any bacterial contamination. This reagent contains 0.05% Kathon CG.
8. **Wash Solution**: Incubate the vial for 15-30 minutes in a water bath at **37°C** until complete dissolution of solids, when present. Shake the vial and dilute the amount required 1:20 in distilled water (the 50 ml contained in the vial allow to prepare 1 liter of Wash Solution). The Wash Solution must be stored at **2-8°C** in its original vial and used within **8 weeks** following opening. The diluted Wash Solution must be used within **7 days**, when protected from any contamination. This reagent contains 0.05% Kathon CG.
9. **TMB substrate:** It is ready to use. When open, it can be used for **8 weeks**, stored at **2-8°C**, and provided that it remains protected from any bacterial contamination.
10. **Stop solution:** It is ready to use.

Cautions: Sulfuric Acid, although diluted to 0.45M, is caustic. As for any similar chemical, handle Sulfuric Acid with great care. Avoid any skin and eye contact. Wear protection glasses and gloves when handling.

Note: Bring the kit at room temperature, at least 30 min. before use. Store the unused reagents at 2-8°C.

The stability studies performed at 30°C show that the reagents keep their performances and can be shipped at room temperature without any damage.

When appropriately used and stored, according to the recommended protocol and cautions, the kit can be used over a two month period, and strip by strip, if required.

PROCEDURE:

Sample collection:

Blood plasma (9 vol.) must be collected on 0.109M (or 0.129M) citrate anticoagulant (1 vol.); plasma supernatant is decanted following a 20 min. centrifugation at 2,500 g; citrated plasma should be tested within **24 hours** or stored frozen at **–20°C** or below for up to 6 months, and thawed for 15 min. at 37°C just before use. Thawed specimen must be tested within **2 hours**.

Tested plasma or sample or control:

Plasma is tested at **1:100** dilution in HIA Sample Diluent (**SD**). When high amounts of heparin-dependent antibodies are expected, samples must be assayed at **1:200** or **1:400** dilution, etc.... Results (corresponding absorbance) must then be multiplied by **2** or **4**, etc....

Controls are ready to use (**already diluted 1:100**).

Assay procedure:

Remove the required number of strips from the aluminium pouch, for the series of measures to be performed. Then put the strips in the frame provided. In the different wells of the micro ELISA plate introduce the reagents and perform the various assay steps as indicated on the following table:

Reagent	Volume	Procedure
CLy	**50µl**	Introduce the **CLy** into the micro ELISA plate wells **(a)**
IgG Positive control or **Negative control** or **1:100 diluted sample** or **sample diluent** (blank)	**200 µl**	Introduce the : – **IgG Positive control** or – **negative control** or – **diluted sample** or – **sample diluent** into the micro ELISA plate wells **(a)**
Incubate for 60 minutes at room temperature (18-25 °C) (b)		
Wash Solution (20 fold diluted in distilled water)	**300 µl**	Proceed to 5 successive washings using the washing instrument **(c).**
Conjugate (anti-IgG (Fcγ)-HRP immunoconjugate, restored with 7.5 ml of conjugate diluent)	**200 µl**	Immediately after the washing, introduce the immunoconjugate in the micro ELISA plate wells**(c)**
Incubate for 60 minutes at room temperature (18-25 °C) (b)		
Wash Solution (20 fold diluted in distilled water)	**300 µl**	Proceed to 5 successive washings using the washing instrument **(c)**.
TMB/H_2O_2 Substrate	**200 µl**	Immediately after the washing, introduce the substrate into the wells. Nota: The substrate distribution, row by row, must be accurate and at exact time intervals **(c,d)**
Let the colour develop for exactly **5 min**. at room temperature (18-25 °C) **(b)**		
0.45M Sulfuric Acid	**50 µl**	Following exactly the same time intervals than for the addition of substrate, stop the colour development by introducing the **0.45M** Sulfuric Acid **(c,d)**
Wait for **10 minutes** in order to allow the colour to stabilize and measure absorbance at **450 nm (A450) (e)**. Substract the blank value.		

Note:

a) Distribute controls and tested specimen as rapidly as possible (within 10 minutes), in order to obtain an homogeneous immunological kinetics for antibodies binding. A too long delay between the distribution of the first and the last wells may induce an influence of immunological kinetics and produce wrong results.

b) Avoid letting the plate in the bright sunlight during incubations and more particularly during colour development. A micro-ELISA plate shaker can be used. An incubation temperature of 18-25°C must be respected. Results are affected by a too high (>25°C) or too low (<18°C) temperature, and measured A450 are then too high or too low. It has to be considered when analyzing the results. In the same way, if a microplate shaker is used, it should be used only at the beginning of each step (sample introduction, immunoconjugate introduction, stop solution introduction), for 1 to 2 minutes, in order to obtain a good homogeneity. A450 values generated in the assay are significantly increased if shaking is used throughout the incubation steps.

c) Never let the plates empty between the addition of the reagents or following the washing step. The next reagent must be added within 3 minutes, in order to prevent the plate from drying, which could damage the immobilized components. If necessary, keep the plate filled with Wash Solution and empty it just before the introduction of the next reagent. The washing instrument must be adjusted in order to wash the plates gently, and to avoid a too drastic emptying, which could lower plate reactivity.

d) For addition of the TMB substrate, the time interval between each row must be accurate and exactly determined. It must be the same when stopping the reaction.

e) For bichromatic readings, a reference wavelength at 690 nm or at 620 nm can be used.

QUALITY CONTROL:

- Controls provided in the kit allow validating the right performance of the assay.
- Expected A450 values for positive and negative controls can present variations from lot to lot but, when the assay is run at room temperature, between 18 and 25°C, they always are:

P = A450 for positive control ≥ 1.0 | **N = A450 for negative control: ≤ 0.25**

Obtained values for P and N, at 20±1°C, are indicated on the flyer provided in the kit.
Obtained A450 can vary according to the effective temperature during the assay run.

EXPRESSION OF RESULTS:

- Results are expressed according to the A450 values, as positive or negative.
- When higher dilutions are used, (i.e. **D**), the complementary dilution factor must be considered.

INTERPRETATION OF RESULTS:

When the assay is run at 20±1°C, the results are as follows:

Positive: **A450 > 0.50**
Weakly Positive: **A450 > 0.30 to < 0.50**
Negative: **A450≤ 0.30**

Note: When the room temperature is out of the recommended range, absorbance values can be affected. The positive control can then be used for adjusting the cut-off value. The flyer provided in the kit indicates the A450 value obtained for the positive control of the ZYMUTEST HIA lot used, and the value in % of this A450 corresponding to the cut-off. The adjusted cut-off value is then the corresponding % of the absorbance measured for the positive control in your series of measurements.

LIMITATIONS OF THE ASSAY:

If the washing step is not correctly performed, the negative control can produce a high absorbance value. In order to avoid non-specific colour development, check that the washing step is performed efficiently.
As for any autoantibody assay, clinical situation such as presence of inflammation, infectious diseases, auto-immune diseases, immun-complexes, can induce a high background, which can be within the grey zone or in the weak positive range. Check then for the possible presence of antibodies on another specimen collected later.
Erroneous results can occur from bacterial contamination of test materials, inadequate incubation periods, inadequate washing or decanting of test wells, exposure of substrate to stray light, omission of test reagents, exposure to higher or lower than prescribed temperature requirements or omission of steps.
The results of this assay should not be used as the sole basis for a clinical decision.
Although a positive reaction obtained using this assay may indicate the presence of a heparin-associated antibody, the detection of such antibodies, however, DOES NOT CONFIRM the diagnosis of heparin-induced thrombocytopenia (HIT).
Some patients may have naturally occurring antibodies to PF4 or other chemokines.

PATHOLOGICAL VARIATIONS:

Heparin dependent antibodies are immunoglobulins present in plasma of patients with suspicion of Heparin-Induced Thrombocytopenia (HIT) type II.
Type II HIT, the immunoallergic type, occurs during heparin treatment [1-2] and remains a major complication of this therapy.
It is caused by the development of antibodies to Heparin-Protein (usually Platelet Factor 4) macromolecular complexes [3-4]. In addition to antibodies to PF4-Heparin, antibodies to other chemokines such as Neutrophil-Activating Peptide or NAP2 and Interleukin-8 or IL8 have also been evidenced in some patients [5].
Development of pathology is mainly associated with heparin-dependent antibodies of the IgG isotype.
However, when the test is used for assessing the risk of developing a clinical complication of HIT, the assay of the global IgGAM isotypes is useful as a prognostic factor for this complication.
When HIT occurs first, inflammation and/or platelet activation mechanisms, associated with various medical or surgical contexts, develop and lead to an increased release of chemokines and then promote formation of heparin complexes with chemokines (usually PF4). These multimolecular complexes can become antigenic and induce the generation of heparin-dependent antibodies. Heterogeneity of these antibodies could partly explain some discrepancies between the clinical suspicion of HIT and biological tests [6].
Frequently, heparin dependent antibodies can be asymptomatic, especially when they are of the IgM isotype. The clinical association is higher with elevated antibody concentrations and with the IgG isotype.

RELATED ASSAYS:

The various isotypes can be measured globally, using the ZYMUTEST HIA IgGAM screening assay kit (# RK040D), for assessments of the risk to develop HIT, in patients treated with heparins: presence of antibodies is a risk indicator for development of HIT.

COMPLEMENTARY CHARACTERIZATION OF POSITIVE SAMPLES (IF REQUIRED):

If required, positive samples can be further characterized by their binding inhibition in presence of heparin. For this confirmation, to 500µl of the 1:100 diluted tested specimen, add 10µl of a 100 IU/ml Unfractionated heparin solution and mix homogeneously. This heparinized solution (2 IU/ml final) must then be tested in the assay. Heparin dependent antibody binding to the plate is then inhibited (decrease in absorbance more than 50%) in almost all the cases. This inhibition confirms the heparin dependent binding of antibodies. In very rare specimen, already positive in the absence of platelet lysate, this inhibition is not observed, and the assay remains positive without or with heparin in the diluent: the result (which remains unclear from the present knowledge) must then be considered as inconclusive, and interpreted along with other assays or criteria for the diagnosis of HIT.

ASSAY SPECIFICITY AND CHARACTERISTICS:

This optimised assay is designed with biologically available and immobilized heparin, then stabilized and saturated, which allows reacting fully with heparin binding proteins and antibodies. This reliable method then provides high reproducibility, by identifying IgG isotype heparin-dependent antibodies, and by mimicking the binding mechanism of antibodies in vivo, on heparin present at the cell surface, especially on platelets or endothelial cells.

INTERFERENCE:

No interference of Heparin up to 1 IU/ml.

PERFORMANCE EVALUATION:

- External study: Zymutest IgG versus Serotonin Release Assay (SRA) for n=174 samples.
  Matches indicate that both were positive or both were negative.

Matches	131
% Matching	75.29

- Two site external study: Zymutest IgG versus Asserachrom for n=243 samples:

		Asserachrom	
		Positive	Negative
Zymutest IgG	Positive	33	17
	Negative	42	151
Agreement		76%	
Co-positivity		44%	
Co-negativity		90%	
Sample Size		243	

- Example of reproducibility data:

	Intra assay			Inter assay		
Sample:	N	A450	CV%	N	A450	CV%
IgG Positive control	6	1.31	3.07	7	1.34	7.11

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Appendix 4: Pretest probability scoring system of HIT: the 'four Ts'

	Points (0, 1, or 2 for each of 4 categories: maximum possible score =8		
	2	1	0
Thrombocytopenia	> 50% fall or platelet nadir 20 – 100 x 10^9/l	30 – 50% fall or platelet nadir 10 – 19 x 10^9/l	Fall < 30% or platelet nadir < 10 x 10^9/l
Timing of platelet count fall or other sequelae	Clear onset between day 5-10; or less than 1 day (if heparin exposure within past 100 days)	Consistent with immunization but not clear (e.g. missing platelet counts) or onset after day 10	Platelet count fall too early (without recent heparin exposure)
Thrombosis or other sequelae (e.g. skin lesions)	New thrombosis; skin necrosis; post heparin bolus acute systemic reaction	Progressive or recurrent thrombosis; erythematous skin lesions; suspected thrombosis but not yet proven	None
Other cause of thrombocytopenia not evident	No other cause of platelet count fall is evident	Possible other cause is evident	Definite other cause is present

Pretest probability score: 6-8 = High; **4-5 = Intermediate**; **0-**3 **= Low** *(reprinted from Warkentin T.E & Heddle N.M. Laboratory diagnosis of heparin induced thrombocytopenia. Current haematology reports 2003).*[48]

Appendix 5: Quantitative interpretation of optical density measurements using PF4-dependent enzyme-immunoassays.[49]

ELISA results (O.D)	LIKELIHOOD OF A POSITIVE SRA
< 0.4	< 1%
0.4 – 1	5%
1 – 1.4	20%
1.4 – 2	50%
> 2	90%

- *Adopted from Warkentin TE, Sheppard JI, Moore JC et al. Quantitative interpretation of optical density measurements using PF4-dependent enzyme-immunoassays. J Thromb Haemost 2008.*
- *SRA – serotonin release assay, is a Gold standard for heparin induced antibody detection.*

Appendix 6: **ETHICAL CLEARANCE LETTER**

KENYATTA NATIONAL HOSPITAL
Hospital Rd. along, Ngong Rd
P.O. Box 20723, Nairobi
Tel: 726300-9
Fax: 72527
Telegrams: MEDSUP", Nairobi
Email: KNHplan@Ken.Healthnet.or

Ref: KNH/UON-ERC/ A/138

29th January 2009

Dr. Msafiri L. Marijani
Dept. of Human Pathology
School of Medicine
University of Nairobi

Dear Dr. Msafiri

RESEARCH PROPOSAL: "HEPARIN ASSOCIATED COMPLICATIONS IN ADULT PATIENTS AT KENYATTA N. HOSPITAL" (P332/12/2008)

This is to inform you that the Kenyatta National Hospital Ethics and Research Committee has reviewed and **approved** your above cited research proposal for the period 29th January 2009 –28th January 2010.

You will be required to request for a renewal of the approval if you intend to continue with the stud beyond the deadline given. Clearance for export of biological specimen must also be obtained fron KNH-ERC for each batch.

On behalf of the Committee, I wish you fruitful research and look forward to receiving a summary o the research findings upon completion of the study.

This information will form part of database that will be consulted in future when processing related research study so as to minimize chances of study duplication.

Yours sincerely

DR. L. MUCHIRI
AG. SECRETARY, KNH/UON-ERC

c.c. Prof. K.M. Bhatt, Chairperson, KNH/UON-ERC
The Deputy Director CS, KNH
The Dean, School of Medicine, UON
The Chairman, Dept. of Human Pathology, UON
Supervisors: Dr. Rajab J. A, Dept. of Human Pathology, UON
Dr. Dave P., Dept. of Human Pathology, UON
Dr. Alexander Duncan, Emory University, Atlanta, USA

Appendix 7: Ethical committee approval letter to change the study title

KENYATTA NATIONAL HOSPITAL
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Email: KNHplan@Ken.Healthnet.org
September 10, 2010

Ref: KNH-ERC/ MOD/775

Dr. Msafiri M. L
Dept. of Human Pathology
School of Medicine
University of Nairobi

Dear Dr. Msafiri

Re: Approval to change title of the dissertation - study titled "Heparin associated complications in adult patients at KNH"(P332/12/2008)

Your letter dated August 2, 2010 refers.

The request of change of title of your dissertation from "Heparin associated complications in adult patients at KNH" to "Heparin induced Thrombocytopenia syndrome in adult patients at KNH" has been considered and approved by the KNH/UON-Ethics and Research Committee.

All the related documents will be amended appropriately.

Yours sincerely

PROF A N GUANTAI
SECRETARY, KNH/UON-ERC

c.c. Prof. K.M. Bhatt, Chairperson, KNH/UON-ERC
The Deputy Director CS,KNH
The Dean,School of Medicine,UON
Supervisors: Dr. Rahab J., Dr. Dave P, Dr. Duncan A.