University of Nairobi

CREATINE PHOSPHOKINASE SERUM LEVELS IN ACUTE CLOSED ISOLATED FEMUR FRACTURES.

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A study submitted in partial fulfilment of the requirements for the award of Degree of Masters of Medicine in Orthopaedic Surgery of the University of Nairobi.

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DECLARATION

I declare that this dissertation/thesis is my original work and has not been submitted elsewhere for examination, award of degree or publication. Where other peoples work or my own has been used, this has properly been acknowledged and referenced as per University Of Nairobi requirements.

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DEDICATION

I dedicate this study to my parents for their continued support throughout my training and to my teachers for their guidance.

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2. To Lancet group of Laboratories for analysing the samples and providing the results efficiently.

LIST OF ABBREVIATIONS

A&E	Accident and Emergency Department
ANCOVA	Analyses of covariance
ANOVA	Analyses of variance
BUN	Blood Urea Nitrogen
CC	Correlation Coefficient
CI	Confidence Interval
CK	Creatine Kinase
CPK	Creatine Phosphokinase
KNH	Kenyatta National Hospital
MIS-AL	Minimally Invasive Anterolateral Hip Replacement
MRI	Magnetic Resonance Imaging
RPM	Revolutions Per Minute
SANAS	South African National Accreditation System
UoN	University of Nairobi
U/S	Ultrasonography

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1. ABSTRACT

1.1 BACKGROUND

Soft-tissue injuries in closed fractures are less obvious than in open fractures, but still have enormous importance. The evaluation of soft tissue injury in closed fractures can be much more difficult than open fractures and the severity is easily underestimated. Of note is that closed fractures are much more common than open fractures, i.e. of all fractures recorded it is estimated only 3% are open fractures. Understanding soft tissue status is important as the effective treatment of any fracture depends upon good soft tissue management. The current methods of soft tissue assessment include clinical examination or use of imaging. Specifically MRI and U/S which are expensive or not readily available. This study aims at establishing the relationship between the pattern of closed fracture femur as defined in the AO classification and severity of soft tissue injury by measuring the creatine phosphokinase levels in serum.

1.2 OBJECTIVE

To measure the serum creatine levels in patients with closed fracture femur as a biochemical marker of degree of muscle injury and correlate this to AO fracture pattern classification.

1.3 STUDY DESIGN

Cross-sectional multi-centre hospital based study

1.4 SETTING

The patients were recruited from two Kenyan hospitals that deal with trauma patients, Kenyatta National Hospital Accident and Emergency department, Orthopeadic wards and Orthopaedic clinic, Kikuyu Mission Hospital.

1.5 METHODOLOGY

Consecutive sampling of patients presenting in KNH with isolated closed fracture femur was done. Their blood samples were collected at 48hrs after the time of injury. The blood samples were transported to Lancet at specified conditions optimum for CK analysis. The CK analysis was done using the COBAS INTEGRA 400/800 whose test principle measures the rate of production of β-Nicotinamide Adenine Dinucleotide which is directly proportional to CK activity. The levels of CK was correlated to fracture pattern as classified in AO classification of shaft fractures.

1.6 DATA PROCESSING

The collected data was coded and analysed using Microsoft Excel, SPSS 20. and represented in the form of percentages, means and their 95% confidence intervals.

The results showed the following, males were more than the females but females were predominant in the group that had pathological fractures. All the patients except one had normal BUN levels. None of the patients had clinical signs of compartment syndrome or ABI less than 0.9.There was a correlation between the mechanism of injury and CK levels, CK levels and urea/creatine ration but no correlation between CK levels and fracture pattern. Also noted were many outliers thought to be due to other contributing factors. Females had a generally lower amount of CK compared to the males. Those who sustained pathological fractures had CK levels within normal.

1.7 CONCLUSION

There is a correlation between mechanism of injury and CK levels, CK and urea/creatine ration. No correlation was noted between fracture pattern and BUN levels or CK and fracture pattern or CK and BUN levels.

1.8 RECOMMENDATIONS

A follow up study is recommended to evaluate whether CK levels would correlate with fracture healing and functional outcomes.

2. BACKGROUND

2.1 INTRODUCTION

The current definition of a fracture is a soft tissue injury with a break in the bone.
And the effective treatment of any fracture depends upon good soft tissue management (53,54,55). Therefore, even closed fractures need a sophisticated management protocol and an excellent grading system to achieve the goal of uncomplicated healing with complete restitution of function.
The degree of injury and tissue ischemia may not be apparent in closed fractures. This can make diagnosis and therapeutic decisions difficult. Many modern imaging techniques permit qualitative assessment of closed soft-tissue injuries, but clinically useful quantitative assessment of damage is not yet available. The imaging modalities commonly used to assess muscle injury are MRI and U/S. In our setting, these are not always readily available and when available the cost may still be unaffordable for many of the patients.
Muscle injury leads to rhabdomyolysis and that one of the systemic effects of rhabdomyolysis is myoglobinuric acute renal failure. It has been noted that even after achieving good alignment and fixation of fractures, some patients are still not able to achieve their previous functional levels in terms of gait and muscle power.
Which leads to the following questions: one, would quantifying the degree of soft tissue injury explain why some patients resume full/almost full normal function while others do not? Secondly, how can this objective quantification be done? Thirdly, what is the incidence of acute renal failure in patients with closed femur fractures in our setting?
Creatine kinase and myoglobin are among some of the most useful markers of muscle injury (33). The level of creatine phosphokinase in serum has been used to assess the operative invasiveness of different approaches used in hip arthroplasty (28). The results of this study showed that there is a significant difference in the levels of CK with MIS-AL compared to standard hip approaches as a consequence of the amount of muscle trauma sustained with each approach.
However, there have been numerous studies on CK, myoglobin and renal failure in trauma patients, but there is currently no study comparing AO classification and CK levels as a marker of degree of soft tissue injury. Secondly there is no local data showing the effect of trauma related rhabdomyolysis on renal function.

This study aimed to correlate the degree of muscle injury sustained in closed diaphyseal fracture femurs by analysing the levels of CPK and correlating this to the different fracture patterns defined on the AO diaphyseal classification system . The study also correlated the levels of CK serum levels to the BUN levels.

2.2 LITERATURE REVIEW

2.2.1 Understanding Creatine Phosphokinase

Creatine kinase is a compact molecule of about 82kDa normally found in two places in the body, the cytosol and mitochondria of tissue where energy demands are usually high. In the cytosol, CK is made up of two peptode chains of about 42kDa, M subunit (muscle) and B (brain). These two polypeptides combine to allow for the formation of three different isoenzymes that are tissue specific: CK-MB (cardiac muscle), CK-BB (found in the brain) and CK-MM (generally found in skeletal muscle). In humans, the distribution of the isoenzymes varies with the type of muscle: skeletal muscle: 98% MM and 2% MB and cardiac muscle: 70-80% MM and 20-30% MB, BB predominates in the brain. The two dominant and specific CK types found in mitochondria are: non-sarcomeric form called ubiquitous Mt-CK found in several tissues like the brain, SM and sperm while the sarcomeric form is found in the heart and skeletal muscle (1).
Historically, measurement of CK levels was essential in the diagnosis of ischaemic disorders of the cardiac muscles in patients presenting with a history of chest pain at the hospistal. Currently, serum CK levels are still generally associated with myocyte damage/ disruption or myocyte affecting disease (2).
Hence, determination of serum CK activity and further quantification of the specific isoenzyme remains a crucial marker of myocyte necrosis and muscle tissue damage due to trauma or disease (3).

2.2.2 Serum Levels

Acceptable normal values of CK in serum in the general population can be between 35-175U/L but occasionally noted to be as high as 16,000u/l with this wide range being thought to be due to asymptomatic cases, minor injury, genetic factors or the level of physical activity an individual participates in and lastly due to known/unknown medicines being consumed.
Whenever a patient has rhabdomyolysis, CK levels are usually found to be quite high, reaching levels between 10,000-200,000U/L. Levels that are that high are considered to an indication of myocyte damage or membrane disruption resulting in release of intracellular components into the blood (8,9).
Still under research is the mechanism through which CK is removed from circulation and it is likely that what is measured reflects a complex interaction associated with energy status and level of striated myocyte damage. Hence, observed serum CK is thought to represent relative amounts of CK leaked , amount of enzyme activity and the rate of removal of CK from the blood (10).
From previous studies, it has been observed that CK starts to increase within 12hours of onset of muscle injury, reaches its peak in 24-72 hours then declines gradually over 72-120hours as long as muscle injury is not continuing (24, 25, 26, 27).

a. *Serum level variations with gender*

There have been reports in literature of gender differences in muscle disturbance and repair processes. Research using female animals has shown that lower baseline levels of CK in females (11).But, even in human beings, there are still marked sex differences in CK serum levels at rest, with higher values in males than in females(34,35).

b. *Serum level variation with age*

The bulk of the muscle and function gradually reduce with age, and cell apoptosis is thought to have a role in age-related sarcopenia (12).

In neonates, CK serum levels are much higher than those in adults and are dependent on gestational age, but the values reach adult levels within the first 10 days of life (35,36).

In older female subjects, lower levels of serum CK have been thought to be due to a decline in circulating neutrophils with age which may, in part, be as a result of reduced oestradiol levels and endogenous antioxidant status (13). Neutrophils in the blood produce oxidants such as superoxide free radicals, which lead to cell damage and leakage. Therefore, an elevated serum CK might be related to optimal functioning of the cell, which may decrease with age, and is not simply a marker of less damage (14).

c. *serum level variation due to ethnic/genetic differences*

Black men usually have higher values than Caucasians and although black men usually have a higher body weight and a denser lean body mass, this does not correlate with CK levels. Generally though some studies do not report any differences in the CK serum values between black and white athletes (37, 38, 39, 40).

d. *serum level variation in relation to body mass*

CK activity is postulated to be related to body mass and physical activity, with resting levels higher in people who actively participate in sports than in sedentary subjects, given the regularexercises that athletes carry out (41, 42, 43).

2.2.3 Myoglobin

Myoglobin is a cytoplasmic hemoprotein made up of a single polypeptide chain of 154 amino acids. It is expressed solely in cardiac myocytes and oxidative skeletal muscle fibers. The molecule was so named because of its functional and structural similarity to haemoglobin (45, 46).

To assess the amount of skeletal muscle damage, plasma CK activity and plasma myoglobin levels have been widely used as markers for muscle injury (47).

Myoglobin is normally bound to plasma globulins, and is maintained at a low serum level of 0 to 0.003mg/dL[3]. Once circulating myoglobin levels have exceeded 0.5 to 1.5mg/dL it overwhelms its protein binding capacity, tubule endocytosis rate and metabolism rate, and is rapidly excreted in the urine (48).

Serum myoglobin levels rise and drop much faster than CK levels (in 1 to 6 hours), thus have a low negative predictive value and may not be used as a ruling out test. Also, myoglobolinuria is not always visible, or may be resolved early; it takes a urine myoglobin level of 100mg/dL to cause tea or cola colored urine (49). Detecting myoglobinuria is commonly done using urine dipstick tests (ortho-toluidine).
Myoglobin related renal failure is thought to involve the formation of casts that obstruct the tubules and renal vasoconstriction involving afferent glomerular arterioles and glommerular capillaries. Back leakage of glomerular filtrate through damaged tubules also occurs. Iron pigments derived from muscle and blood have also been shown to play a role in catalyzing the formation of free oxygen radicals and perhaps in inhibiting the vasodilatation effect of endothelium derived relaxing factors in the kidney (51).
Therefore alkalinisation of urine in patients with rhabdomyolysis is protective, presumably because haemoglobin and myoglobin are more soluble in an alkaline solution and the formation of casts is therefore prevented (52).

2.2.4 Soft tissue injury in closed fractures

In closed injuries, the degree of injury and ischemic tissue may not be apparent and this can make diagnosis and therapeutic decisions difficult (15). A classification of the soft-tissue injury should consider all essential factors and guide treatment. It effectively decreases complications by preventing avoidable treatment errors and should be of some prognostic value.

The current classifications of soft tissue injury are mostly subjective hence likely to have intra-observer and inter-observer variability. One of these is shown below:

Tscherne classification of closed fractures (16)

- **Closed fracture grade 0 (Fr. C 0):** There is no or minor soft-tissue injury with a simple fracture from indirect trauma. A typical example is the spiral fracture of the tibia in a skiing injury.
- **Closed fracture grade I (Fr. C 1):** There is superficial abrasion or skin contusion, simple or medium severe fracture types. A typical injury is the pronation-external rotation fracture dislocation of the ankle joint: The soft-tissue damage occurs through fragment pressure at the medial malleolus.
- **Closed fracture grade II (Fr. C 2):** There are deep contaminated abrasions and localized skin or muscle contusions resulting from direct trauma. The imminent compartment syndrome also belongs to this group. The injury results in transverse or complex fracture patterns. A typical example is the segmental fracture of the tibia from a direct blow by a car fender.
- **Closed fracture grade III (Fr. C 3):** There is extensive skin contusion, destruction of muscle or subcutaneous tissue avulsion (closed degloving). Manifest compartment syndrome and vascular injuries are included. The fracture types are complex.

It is well known that the pattern of fracture sustained on the bone is related to the amount of energy involved. Fractures of the femoral shaft are markers of high-energy injuries and since the femur is well enveloped by muscles, theoretically speaking some of the energy is absorbed by the soft tissue envelope (18, 19, 20, 21).
Fractures can be caused by direct or indirect forces. Indirect trauma usually involves less energy than a direct blow and causes proportionately less fragment displacement and soft tissue damage (22). The differing injury patterns are recognized in the various AO classifications (23).

The mechanism of soft tissue injury

i. Direct impact
ii. Implosion
iii. Displacement during movement
iv. Inflammatory process
v. Ischaemia due to blood supply interruption, oedema or compartment syndrome.

I, ii, iv not much can be changed but as for iii early splintage (adequate splintage done immediately at site of injury) could help reduce the secondary injury due to fragment motion. With compartment syndrome, diagnosis is clinical and use of compartment pressure measurements. The equipment is not readily available locally hence the reliance of clinical examination with confirmation requiring emergency fasciotomy to be done. In the case of vascular injury, arteriography is the gold standard but this is expensive and not always available. Hence the use of Ankle Brachial Index.

Ankle brachial index is a reliable, rapid, non-invasive tool for diagnosing vascular injury in the lower limbs. In their study, Mills et al concluded that the positive predictive value, sensitivity and specificity of an ABI lower than 0.90 were 100%. The negative predictive value of ABI that reached 0.90 was also found to be 100% (4).

2.2.5 The fracture patterns based on AO classification (22)

Spiral (A1) and spiral wedge (B1) fractures result from indirect rotational forces. They have large areas of bone surfaces in contact, and minimal soft-tissue damage.

Oblique wedge fractures (B2) are produced by bending forces. The force applied to the limb is considerable and the resulting damage to soft tissue and periosteum is significant.

Transverse fractures (A3), fragmented wedge fractures (B3) and complex fractures (type C) are usually caused by direct forces which are often enormous, especially in the femur. If the bone is of normal quality and the fracture is widely displaced, the degree of soft-tissue damage will be extensive. Even with intact skin, direct exposure of these fractures results in further insult to the soft tissues.

Therefore fracture type and displacement are good predictors of soft-tissue damage. This insight should guide the surgeon towards suitable methods of reduction and fixation. The greater the anticipated soft-tissue damage, the more important the timing of surgery and the choice of approach, reduction technique and implant.

Other Methods currently used to assess soft tissue injury

The development of electronic engineering has led to the construction of diagnostic machinery able to identify the finest details of the muscle. The use of these devices in muscular injury diagnostics has acquired fundamental importance in Medicine as regards diagnosis, prognosis and rehabilitation. Whether to use Ultrasound or MRI is still subject to debate as it is influenced by several factors, such as type of injury, availability of the devices, the Radiologist's knowledge of muscle injuries, and the cost-benefit ratio.
Ultrasound and MRI play an important role in the study of muscle injuries owing to their ability to identify lesions effectively, which is closely related to the presence of oedema in the damaged muscle.

MRI is regarded as the gold standard however, in one study MRI correctly detected the muscle oedema in seven of their patients with DOMS, but the pattern was non-specific and the correct diagnosis required integration with laboratory findings such as creatine kinase assay (5).

2.2.6 Creatinine kinase and Trauma/Orthopaedics

Strecker et al concluded that the amount of fracture and soft-tissue damage can be estimated early by analysis of serum interleukin-6 and creatine kinase and is of great importance with regard to long-term outcome after trauma (29).

More recently creatine kinase levels in serum have been used to provide objective assessment of degree of local soft tissue injury during hip replacement surgery. In summary these have been the findings;

1). levels of serum CK was lower in MIS-AL compared to standard hip procedures (28).

2).Levels of serum CK in patients treated with minimally invasive total hip replacement through a Smith Peterson approach had lower levels compared to those done through a posterior approach (30).

3). Use of minimally invasive anterior approach caused less pain, but higher postoperative levels of CK, than the use of direct lateral approach(31).

The assumption is that the procedure with lower levels of serum CK has less muscle injury either due to less muscle dissection or less retraction of muscles during the procedure (32).

2.3 STUDY JUSTIFICATION

Soft-tissue injuries in closed fractures are less obvious than in open fractures, but still have enormous importance. Their evaluation can be much more difficult than open fractures and their severity is easily underestimated. Yet, the effective treatment of any fracture depends upon good soft tissue management. Currently there is no study correlating the AO fracture classification to CK serum levels as marker of soft tissue injury. Secondly, there is no data showing the state of renal function in trauma patients locally.
It is unknown whether biochemical markers and the impact of soft-tissue trauma correlate.(ref paper)But it is known that CK serum levels in trauma patients correlates with renal failure, although we do not have data from a study done locally. It is also known, that a significant no of patients with fracture femur even after well done ORIF, have some residual dysfunction. Could this be associated with the degree of soft tissue injury?? This study aimed at establishing the relationship between the pattern of closed fracture femur and severity of soft tissue injury by measuring the creatine phosphokinase levels in serum.

2.4 STUDY QUESTION:

Is there appreciable correlation of CK serum levels and AO classification of fracture patterns?

2.4.1 PRIMARY OBJECTIVE

To determine the correlation between the fracture pattern as per the AO classification of diaphyseal fractures of the femur and CK serum levels as a biochemical marker of soft tissue injury.

2.4.2 SECONDARY OBJECTIVES

a. To correlate the levels of serum creatine kinase levels with BUN levels
b. To correlate the levels of BUN to the fracture pattern**.**

2.5 HYPOTHESIS

The levels of serum creatine kinase do not correlate to the AO pattern of fractures in closed fractures of the femur.

3.0 METHODOLOGY

3.1 STUDY DESIGN

Multi-centre hospital based cross-sectional study

3.2 STUDY SITE

This study was conducted at the A&E department, orthopaedic ward and orthopaedic clinic in Kenyatta National Hospital and Kikuyu Mission Hospital.

KNH is one of the main teaching and referral hospitals based in Nairobi. Receives referral patients from the entire country for both primary and specialised management. The other hospital is one centre where the University of Nairobi, Department of Orthopaedics has residents rotating as part of their training program. Trauma patients are first received in triage at the A&E department, stabilised then sent for further evaluation and management to the surgical room in A&E. The sitting orthopaedic surgeon reviews the patient and plans the management. If a patient requires surgery, which is the case with fractures of the femur, the patient is admitted and taken to the orthopaedic ward.

3.3 STUDY POPULATION

All the patients presenting to the two mentioned hospitals with isolated acute closed fracture femur. Both male and female between ages of 18-65 years.

INCLUSION CRITERIA	EXCLUSION CRITERIA
Isolated closed fracture femur	Injured during sports
	Known muscle disorders
	Cardiac disease, cardiac trauma
	Huntington disease, multiple sclerosis, and amyotrophic lateral sclerosis.
	Renal failure
	Hypoxic brain disease

	Any patient requiring CPR
	Patients already operated on

3.3.1 RECRUITMENT PROCEDURE

The patients were identified on admission at the surgical room in A&E department. Once stabilised and noted to have only a fracture femur, the patient was explained to what was required, the time and date of injury recorded. 48hours after the estimated time of injury the patient was reviewed, if not yet had surgery, they were recruited at this point while in the ward at this point in time. Once consent was given, the principal investigator or research assistant proceeded to obtain the blood sample and record the pattern of fracture on the chart in appendix II. Of note is that some of the patients were recruited on admission, if at the time of presentation 48hours from time of injury had elapsed. In summary the patients were recruited at 48hours from the time of injury whether it was at the point of admission or already in the ward as long as they satisfied the required criteria.

3.4 DATA & BLOOD SAMPLE COLLECTION

Data was collected by the principal investigator or the research assistant once consent had been obtained using the attached questionnaire.
The blood samples were also collected by the principal investigator or research assistant at 48hrs from injury based on the peak and half life of CK (24-28). The required amount the laboratory manager had recommended to be collected was a minimum of 3mls per patient.
Collection precautions

a. *Use of 20-22 gauge needle*
b. *Blood was drawn from the antecubital region of the arm*
c. *The puncture site was warmed*
d. *Tourniquet use for less than one minute*
e. *Allowed the alcohol wipe to dry*
f. *The syringe plunger was pulled gently during withdrawal and pushed gently during transfer to specimen bottle*
g. *Specimen was not collected from any catheters or lines*
h. *Tubes were filled to the correctly specified volume*

Specimen Transport Conditions: The specimen was transported immediately to Lancet laboratory at room temperature (20-25 degrees).

3.5 LABORATORY PROCEDURE

The specimen was analysed at Lancet Kenya Limited, Upperhill Nairobi Laboratory. Co.Reg. No.: C168507
Facility Accreditation Number: M0462. This is a South African National Accreditation System accredited laboratory provided all SANAs conditions and requirements are complied with. (for more details check appendix VI)
The machine: **COBAS INTEGRA 400/800**
Test principle*:* In the reaction, the CK catalyzes the transfer of a phosphate group from the creatine phosphate substrate to adenosine diphosphate (ADP). The subsequent formation of adenosine triphosphate (ATP) is measured through the use of two coupled reactions catalyzed by hexokinase (HK) and glucose-6-phosphate dehydrogenase (G6PD) which results in the production of ß-Nicotinamide Adenine Dinucleotide (reduced form) (NADH) from ß-Nicotinamide-Adenine Dinucleotide (NAD). The system monitors the rate of change in absorbance at 340 nm over a fixed time interval. The rate of change in absorbance is directly proportional to the activity of CK in the sample.

Creatine phosphate +ADP → creatine +ATP

ATP+ D-glucose ----HK-------- → ADP+ G6P

G6P + $NADP^+$ ------G6PDH ---------→ D-6-phosphogluconate+NADPH+H^+

Specimen collection and preparation:
Collectected serum using standard sampling tubes → free from hemolysis
Plasma using Li-heparin plasma→ free from hemolysis
Stability: 2days at 15-25 degrees celsius
7days at 2-8 degrees celsius
4 weeks at (-15)-(-25) degrees celsius
The samples containing precipitate were then centrifuged before assay was done. Centrifugation was done at 400rpm for 10minutes.
COBAS INTEGRA analyzers automatically calculate the analyte concetration of each sample.
Conversion factor: U/L * 0.0167= ukat/L
Quality control:

Reference range	Precinorm U or Precinorm U plus
Pathological range	Precipath U or Precipath U plus
Control interval	24hrs recommended
Control sequence	User defined

Measuring range:
7-2000U/L (0.12-33.4ukat/L)
Determines samples having higher concentrations via the rerun function.
The analyser can accommodate 100samples at a go.
For more details on the laboratory procedure and machine, check appendix V.

BUN: Was also measured at Lancet laboratory using the cobas integra 400.

3.6 ANKLE BRACHIAL INDEX MEASUREMENT PROCEDURE

- Patient supine, without the head or any extremities dangling over the edge of the table
- The blood pressure cuff was inflated proximal to the artery in question until the pulse in the artery ceases.
- The blood pressure cuff was then slowly deflated until the pulse was redetected, the pressure cuff at that moment indicated the systolic pressure of that artery
- The higher systolic reading of the left and right arm brachial artery was used.
- The pressure in each foot's Posterior Tibial artery and Dorsalis Pedis artery are measured with the higher of the two values used as the ABI for that leg.
- P_{Leg}/P_{Arm} A value less than 0.9 would warrant ordering of an arteriogram

3.7 SAMPLING & SAMPLE SIZE

All the patients with isolated closed fracture femur between the ages of 18yrs-65yrs presenting at KNH and Kikuyu Mission Hospital A&E departments, orthopaedic wards or clinics who meet the inclusion criteria were recruited until the required sample size was achieved.

How the sample size calculation method was chosen (48)

This study aimed to establish whether there is a correlation between serum creatine kinase levels as a biochemical marker of degree of soft tissue injury and the different patterns of fractures sustained in closed femur fractures.
A common measure of association between two variables x(fracture pattern) and y(serum creatine kinase levels) is the bivariate Pearson correlation coefficient ρ(x,y)that characterizes the strength and direction of any linear relationship between x and y. When variables are correlated, knowledge of one allows estimating (predicting) the other.
In cross-sectional correlational research, the x variable may measure exposure to some experience (trauma resulting in fracture of the femur) while the y variable may measure some subsequent behaviour or outcome (muscle injury with release of CK in serum).
The Pearson correlation coefficient ρ(x,y) describes the strength and direction of an assumed linear relationship between x and y. If one variable increases (or decreases) as the other increases (or decreases), then the coefficient is positive (or negative). The strength of a relationship is indicated by the numeric value of the coefficient, which can take a range of values from +1 to -1. Where +1 means directly related, -1 means inversely related and 0 means no linear relationship between the two variables.
A correlation coefficient (CC) that characterizes the entire population is denoted by ρ(x,y), while a CC evaluated for a particular sample of size N is denoted by r(x,y). Accuracy for a given sample size measures how close a measured **r** is to the true population size **ρ** while precision as measured by the standard deviation σ_r improves as the sample size increases.
A two-sided confidence interval (CI) for the Pearson correlation coefficient ρ is an observed range of values that consists of a lower limit (LO) and an upper limit(UP), within which the true value of ρ is found with a specified probability (Corty, 2007; Field, 2009). Consider that a CC is determined as CC = (r +/-σr) for a certain sample N. The CI provides an estimate of the unknown ρ value, and also indicates the reliability of the estimate. A 95% CI would capture the true value of ρ with 95% level of confidence, within lower (LO) and upper (UP) limits:

$$LO = r - 1.96\sigma_r$$
$$UP = r + 1.96\sigma_r$$

The total CI width will be here denoted by CI2w, (CI2w = UP - LO), and the CI half-width by w. The z-score multiplier 1.96 is used to define the 95% CI of a normal distribution.
The final equation adopted is:

$$N = \frac{3.84(1-r^2)^2}{\mathrm{w}^2} + 1 + 6r^2$$

Where w is:

$$\mathrm{w} = 1.96\frac{(1-r^2)}{\sqrt{(N-1-6r^2)}}$$

The CC and its standard deviation σr for N measurements of x,y
data pairs may be computed using standard statistics programs. In this study the computer program used was derived from the above formula. (see appendix V for the program). Therefore, estimating this correlation coefficient to be 0.95, at 95% confidence with 5% level of precision you will require to recruit **66** patients with closed femur fractures.

3.8 STUDY LIMITATION

The serum levels of creatine kinase measured did not differentiate the isoenzymes of creatine kinase.

CK is known to peak between 48-72hrs after injury, which was about the time period the investigator targeted to collect the blood specimen. This was estimated from time of injury as reported by the patient. Therefore, patient memory or illiteracy may affect the accuracy of specimen collection time.

For patients not known to have any of the diseases that cause elevated levels of creatine kinase, there was no confirmatory tests done as part of the study but they may be carried out as part of overall management of the patient.

3.9 ETHICAL APPROVAL & CONSENTING

Approval to conduct the study was sought from the University of Nairobi as well as Kenyatta National Hospital, Ethics and Research Committee (KNH/UoN-ERC). Approval was also obtained from the Ethics and Research Committee at the other hospital. Data collection commenced once this approval was granted.

Participants in this study or their next of kin had to give a written informed consent before being recruited. The consent sought enabled the principle investigator to take the patient's bio-data details, as well as history related to the presenting illness.

Participants were informed that they would not benefit directly in this research but that the results obtained may help improve the approach of management of closed femur fractures. The principal investigator covered the cost for conducting the CK serum levels, BUN and creatine only; any other cost required in the management of the patients was to be covered by the patient.

Participation in this study was purely voluntary in nature and as such, it was clarified to the participants that they would be free to participate or even withdraw their participation at any point during the study without any explanation. Withdrawal of participation would not affect the participant's treatment or management in any way whatsoever. Any patient noted to have acute renal failure from the results obtained was treated accordingly in consultation with the renal physicians.

All information obtained has been treated with utmost confidentiality. The questionnaire is only accessible to the principal investigator. The results from Lancet was collected only by the principal investigator.

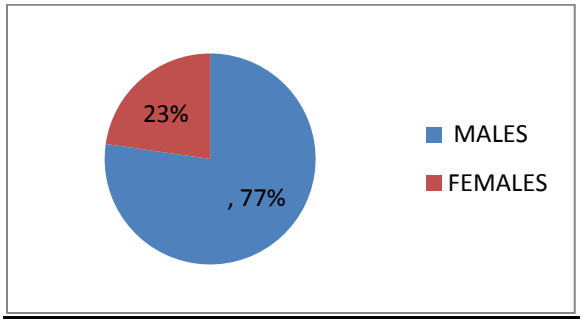
4. DATA ANALYSIS

Data was analysed using excel office for windows 2010 and SPSS 20.0

DATA DEMOGRAPHICS- GENDER DISTRIBUTION TABLE 1

MALE	51	77.27%
FEMALE	15	22.75
TOTALS	66	100%

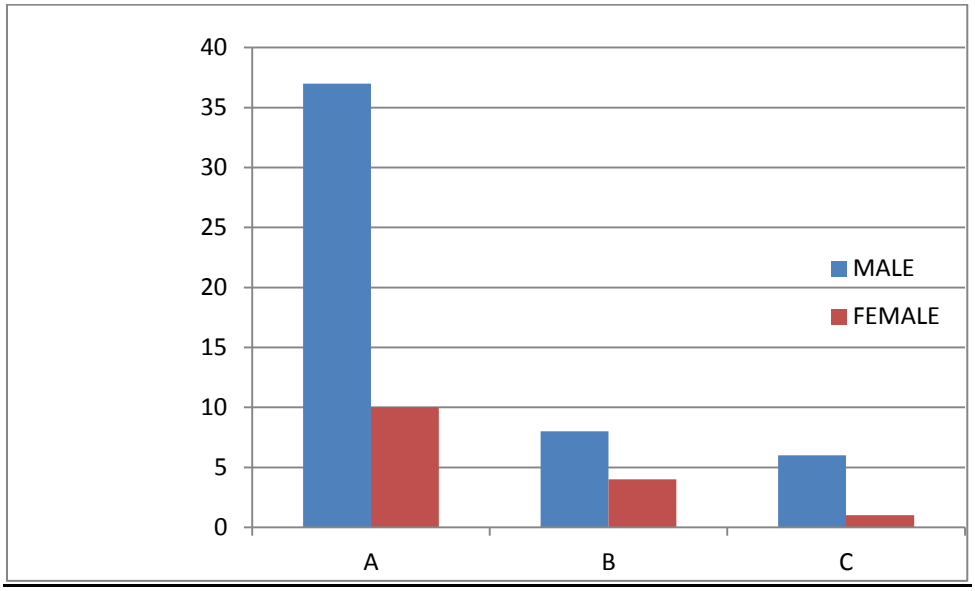
PIE CHART REPRESENTATION OF DATA DEMOGRAPHICS- GENDER DISTRIBUTION FIGURE 1



FRACTURE SEVERITY IN THE TWO GENDERS TABLE 2

FRACTURE SEVERITY	MALE	FEMALE
A	37	10
B	8	4
C	6	1

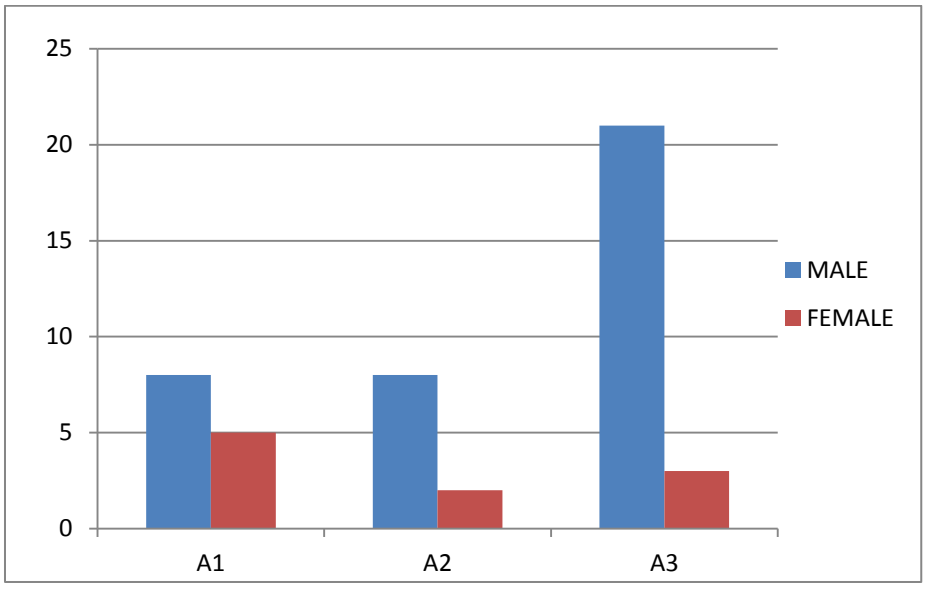
FRACTURE SEVERITY DISTRIBUTION AS PER GENDER FIGURE 2



DETAILED ANALYSIS OF THE A TYPE FRACTURE SINCE IT CONSISTED OF THE MAJORITY TABLE 3

	MALE	FEMALE
A1	8	5
A2	8	2
A3	21	3

HISTOGRAM REPRESENTATION OF GENDER DISTRIBUTION IN THE A TYPE FRACTURE FIGURE 3



CK LEVELS PLOTTED AGAINST GRADED MECHANISM OF INJURY TABLE 4

TYPE A	TYPE B	TYPE C
1	2	3
529	3713	3340
42	1170	8267
119	966	13453
3682	5887	3274
792	2185	5149
117	1602	3420
346	1499	4143
1049	2565	4060
868	4054	3311
1193	536	5943
238	1208	4002
1347	1254	1241
864	7715	776
1950	6780	3998
1942	2423	952
810	189	812
170	6276	11000
	2601	4185
	6937	4441
	2886	
	57	
	8663	
	892	
	7801	
	1345	
	1027	
	3282	
	1540	

CK LEVEL MEANS CALCULATION TABLE 5

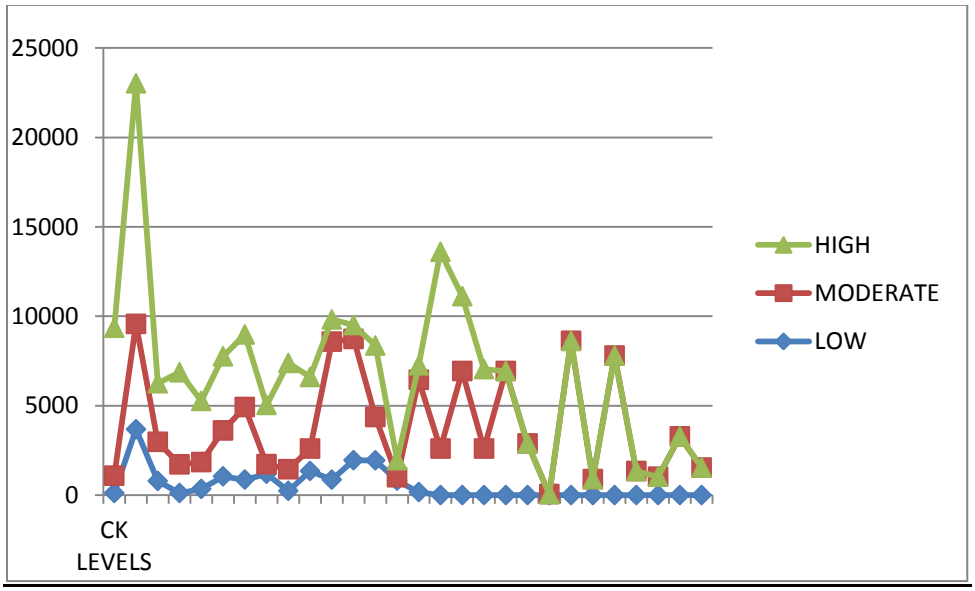
One-Sample Statistics

MOI	N	Mean	Std. Deviation	Std. Error Mean
LOW	17	944.5882	921.91540	223.59733
MOD	28	3109.0357	2601.45780	491.62931
HIGH	19	4514.0526	3300.90611	757.27980

Sample Test

	Test Value = 0					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
LOW	4.225	16	.001	944.58824	470.5831	1418.5934
MOD	6.324	27	.000	3109.03571	2100.2957	4117.7757
HIGH	5.961	18	.000	4514.05263	2923.0668	6105.0385

CK LEVELS PLOTTED AGAINST GRADED MECHANISM OF INJURY FIGURE 4

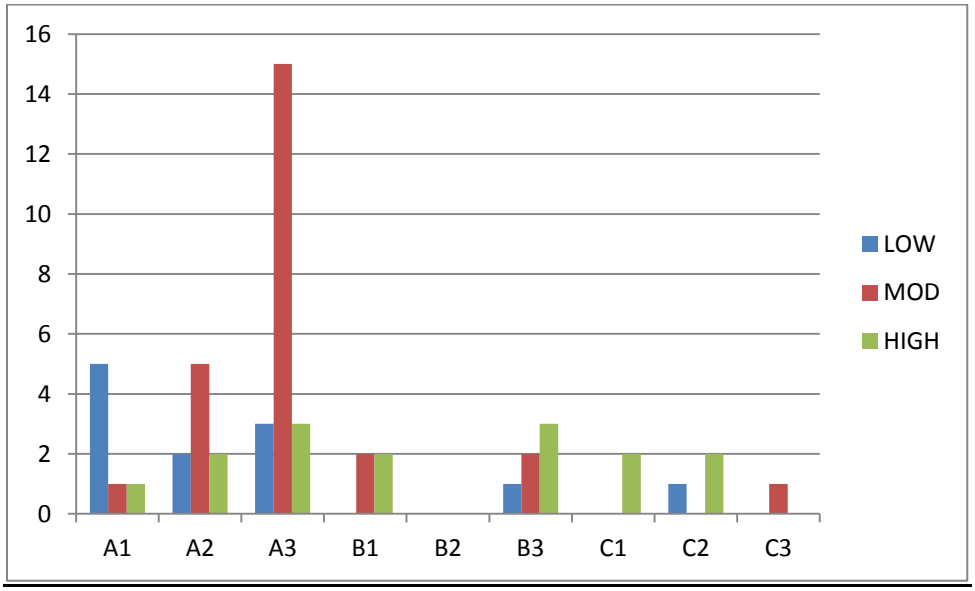


GRADED MECHANISM OF INJURY PLOTTED AGAINST FRACTURE PATTERN TABLE 5

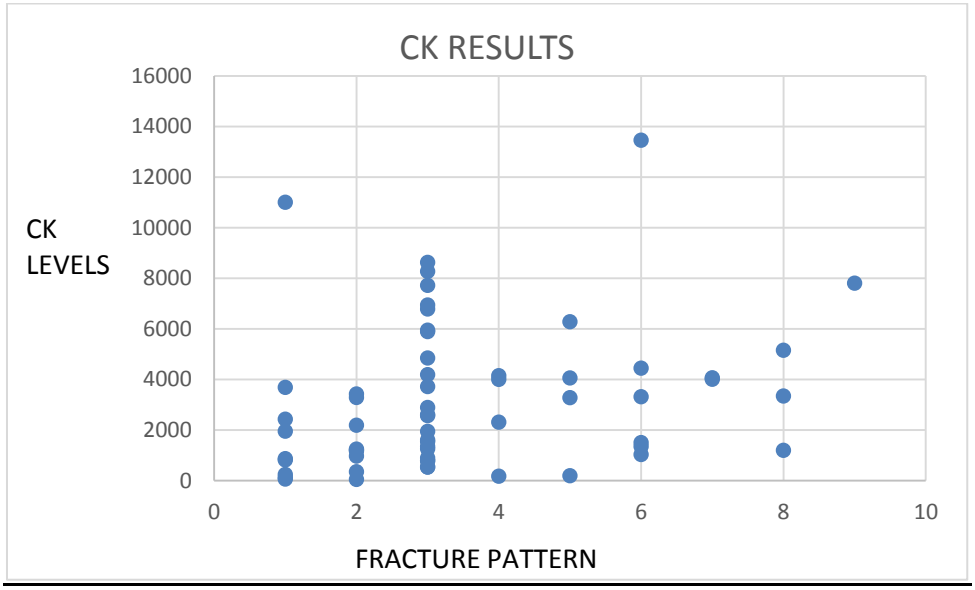
FRACTURE PATTERN

G. MOI	A1	A2	A3	B1	B2	B3	C1	C2	C3
LOW	5	2	3	0	0	1	0	1	0
MOD	1	5	15	2	0	2	0	0	1
HIGH	1	2	3	2	0	3	2	2	0

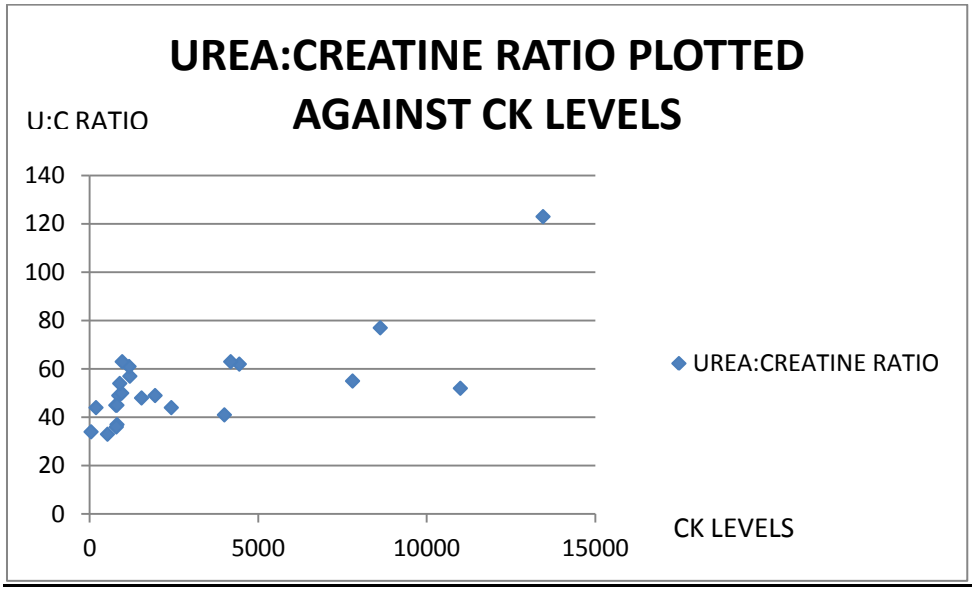
GRADED MECHANISM OF INJURY PLOTTED AGAINST FRACTURE PATTERN FIGURE 5



FRACTURE PATTERN PLOTTED AGAINST CK LEVELS FIGURE 6



UREA:CREATINE RATIO PLOTTED AGAINST CK LEVELS FIGURE 7



CORRELATION BETWEEN CK LEVELS AND FRACTURE PATTERN TABLE 6

		CK	FRACPAT
CK	Pearson Correlation	1	-.058
	Sig. (2-tailed)		.638
	N	66	66
FRACPAT	Pearson Correlation	-.058	1
	Sig. (2-tailed)	.638	
	N	66	66

*. Correlation is significant at the 0.05 level (2-tailed). 95% Confidence interval

CORRELATION BETWEEN UREA/CREATINE RATIO AND CK LEVELS TABLE 7

		UCRATIO	CK
UCRATIO	Pearson Correlation	1	.242*
	Sig. (2-tailed)		.048
	N	66	66
CK	Pearson Correlation	.242*	1
	Sig. (2-tailed)	.048	
	N	66	66

*. Correlation is significant at the 0.05 level (2-tailed). 95% confidence interval

CORRELATION BETWEEN CK LEVELS AND GRADED MECHANISM OF INJURY TABLE 8

		CK	GMOI
CK	Pearson Correlation	1	-.175
	Sig. (2-tailed)		.158
	N	66	66
GMOI	Pearson Correlation	-.175	1
	Sig. (2-tailed)	.158	
	N	66	66

*. Correlation is significant at the 0.05 level (2-tailed). 95% confidence interval

CORRELATION BETWEEN UREA AND FRACTURE PATTERN TABLE 9

		UREA	FRACPAT
UREA	Pearson Correlation	1	.078
	Sig. (2-tailed)		.532
	N	66	66
FRACPAT	Pearson Correlation	.078	1
	Sig. (2-tailed)	.532	
	N	66	66

*. Correlation is significant at the 0.05 level (2-tailed). 95% confidence interval

RESULTS

A total of 66 patients who had fracture femur were recruited and blood samples taken for CK levels. Of the 66, 15 were females and 51 were males. Females -22.7%, Males -77.27%. (Table 1)

Only one patient had elevated levels of urea and creatine slightly above the normal range which resolved with adequate hydration.

Of the fracture patterns, A3 (transverse) was the most common sustained by the patients recruited (21 of 66), TABLE 2 and 3 and FIGURE 2 and 3. Of note is that majority of the patients who sustained A1 (spiral) fracture pattern were females(6 of 7), TABLE 3 and FIGURE 3. Majority of the type A1 fracture pattern were also pathological fractures.

Females had a generally lower level of CK levels compared to their male counterparts even with similar fracture patterns. At the same time, patients who were more muscular had a higher CK level compared to less muscular patients.

Mechanism of injury associated with direct hit or crush to the muscles e.g caved in by soil or bumper hit to the limb were generally associated with higher levels of CK, FIGURE 4. Mechanism of injury appears to have a better correlation with CK levels compared to the fracture pattern. There is also a significant correlation between the urea/cratine ratio and CK levels as demonstrated by FIGURE 7 and TABLE 7 showing a correlation of level of <0.05 as calculated through Pearson's correlation.

However the Pearson's correlation calculated for the correlations between CK levels and fracture pattern, CK levels and graded mechanism of injury and Urea and fracture pattern yielded a non-significant level of more than 0.05. Although noted that the mean differences calculated for the CK levels for the three main groups of fractures was found to have a difference that was significant, 0.01.

DISCUSSION

In closed injuries, the degree of injury and ischemic tissue may not be apparent and this can make diagnosis and therapeutic decisions difficult (15). The purpose of this study was to evaluate whether there is a correlation between CK serum levels and fracture pattern as classified by AO classification of fractures. CK serum levels was used as a marker of muscle injury (8,9). AO classification was used as each pattern of fracture in the classification represents the expected amount of energy involved in the injury (22). Isolated femur fracture was used following the argument that fractures of the femoral shaft are markers of high-energy injuries and since the femur is well enveloped by muscles (18, 19, 20, 21).

Normal expected levels of serum CK in general population varies between 35–175 U/L. (6). In this study the laboratory range for CK provided by the laboratory was up to 184U/L. Elevated

levels clearly represent a strong disruption of striated muscle tissue with concomitant release of intracellular muscle components into the circulation (8, 9).

It is well known that there are still marked sex differences in human beings in CK serum levels at rest, with lower values in females than in males (34,35). In this study it was also noted that the CK levels even when elevated was still lower in females with similar fracture patterns compared to the male patients. One of the explanations for this could be due to the muscle bulk in females being lower than in males (41). The second reason could be related to the mechanism of injury, female patients tend to be involved in lower energy accidents compared to the male patients (56). However, it is notable that the majority of female patients in this study were >50 hence probability of insufficiency fractures was higher which would also explain the low energy fractures hence, low CK serum levels and the fracture pattern sustained (57).

It is well known that the pattern of fracture sustained on the bone is related to the amount of energy involved. Fractures of the femoral shaft are markers of high-energy injuries and since the femur is well enveloped by muscles, theoretically speaking some of the energy is absorbed by the soft tissue envelope (18,19,20, 21). However, in this study the levels of CK did not match very well with the fracture pattern but there was a definite correlation between the CK levels and mechanism of injury. Patients who sustained high energy injury or direct force/crush injury type to the thigh/femur had higher levels of CK regardless of the fracture pattern sustained. The authors thought this could be explained by assuming that in those fractures of lower grading as per AO that had higher levels of CK than expected, the reason could be because most of the force involved was absorbed by the muscle envelope therefore, less of the energy reached the bone to cause a higher grade fracture pattern. As already defined earlier a fracture, is a soft tissue injury with a break in the bone (22). The other reasons could be related to mechanism of soft tissue injury such as direct impact, implosion, displacement during movement, degree of fracture displacement, inflammatory process, ischaemia due to blood supply interruption or oedema. These reasons which the investigator did not have control of could explain the reason for the mismatch between CK levels and fracture pattern. It is important to note that none of the patients had signs of compartment syndrome clinically or vascular injury as checked by measuring the ABI (4). The other factor to consider that may explain the mismatch was the fact that all this patients received intravenous fluids as part of their initial resuscitation measures. The effect of intravenous fluid on the levels of CK and BUN could have been through dilution or increased clearance through the kidneys (58). Also to be considered is whether all the CK released from the injured muscles actually leaked into circulation.

Patients who sustained pathological fractures had their CK levels within normal range. The mechanism of injury in these patients was indirect rotational forces which seemed to be transmitted through bone and muscle but was not sufficient to cause muscle injury and only caused a break in the bone at an area of weakness (59).

CONCLUSION

It was noted that:

1. There was a more clear correlation between mechanism of injury and CK levels as compared to CK levels and fracture pattern.
2. There was also a significant positive correlation between urea/creatine ration and CK levels.
3. Females had lower CK level elevation in general even after trauma could be related to the muscle bulk or mechanism of injury.

Recommendations: a follow up study that would include larger numbers, capacity to assess for muscle bulk, an objective way to measure the amount of forces absorbed by the soft tissue envelope, long term follow up of the patients to assess for bone healing and functional outcome.

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6.0 APPENDIX

Appendix I Questionnaire

Biodata

Hospital no. : **Study No. :**

X-ray No. :

Date and time of specimen collection:

Patients Details

1. **Age:** Years..... Months.....
2. **Sex:** Male..... Female.....
3. **Date of Injury** ...../...../.....(day/month/year)
4. **Time of Injury** ..... : .....(12hr system)

Mechanism of Injury

1. MVA

Passanger

Pedestrian

2. MCA

On the Morocycle

Pedestrian

3. Fall from height.....(in meters)

4. Crush Injury.....(exact object)

5. Assault.....(exact object of assault)

Injury Details

1. Signs of Compartment syndrome Y.....N.....

2. Presence of other fractures Y .....N.....

Site .....

3. Presence of other areas of soft tissue injury Y.....N.....

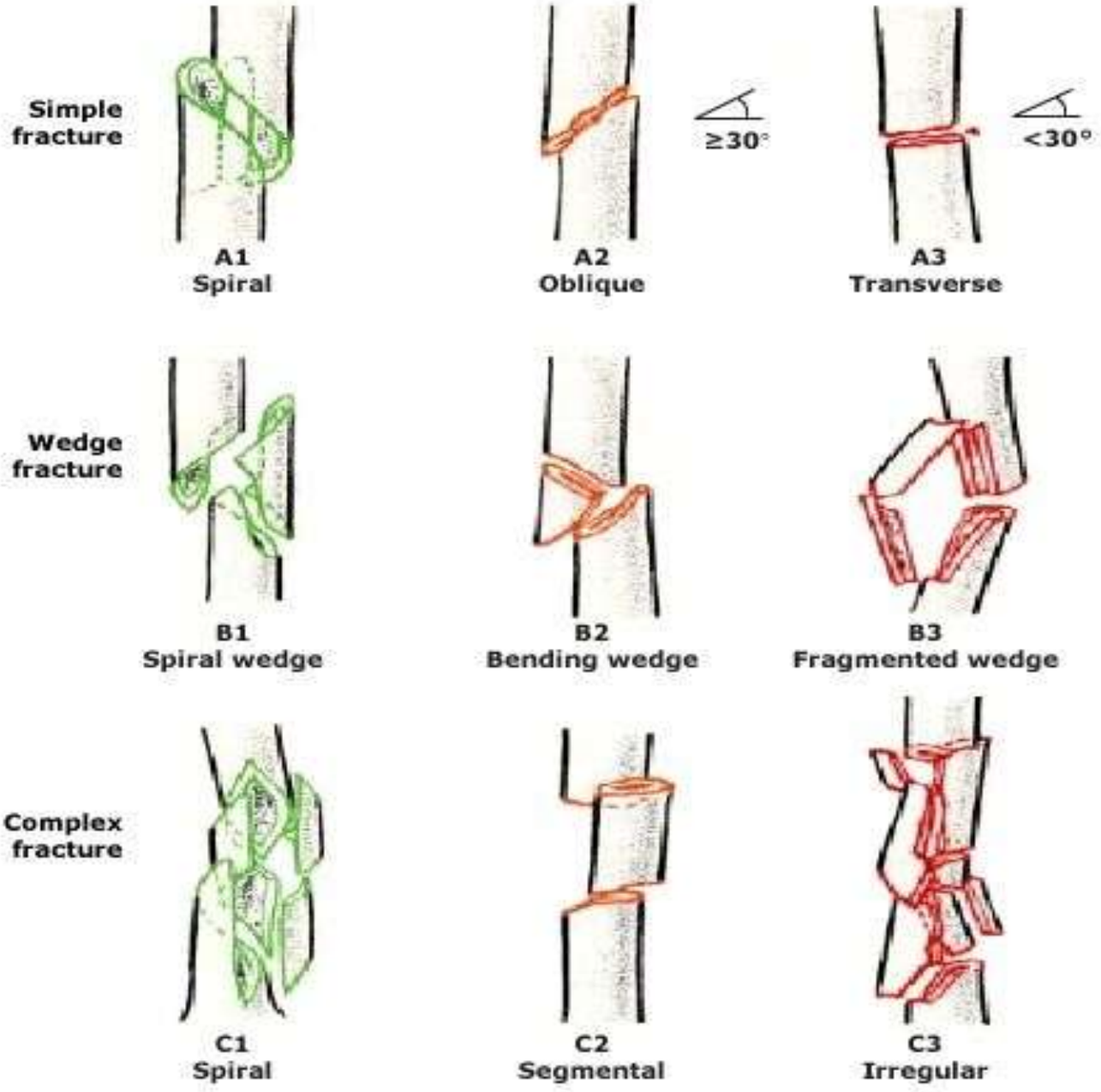
Site.....

4. ABI Measurement (Ankle Brachial Index**).....**

5. Did the patient receive any intramuscular injection Y.....N.....

Appendix II AO Fracture Classification

Fracture Classification - Adopted from Muller AO Classification of diaphyseal fractures. *Circle appropriately*



RECORDED FRACTURE PATTERN:

Appendix III Consent to Participate in a Research Study

Principal investigator: Dr. Dorcas Warigia Chomba, Department of Orthopaedics, University of Nairobi

Research Title: Creatinine Phosphokinase serum levels in acute closed isolated femur fractures.

Brief description on the research study: The study aims to measure the serum creatine phosphokinase as a biochemical marker of severity of soft tissue injury levels in patients with isolated femur or isolated tibia fractures. The serum CK levels with be correlated to the pattern of fracture sustained.

What is expected of the patient and the informant if it's not the pateint

Once the patient has been stabilised and fully evaluated by the attending doctor, the principal investigator or research assistant will explain to the patient about the study and what it entails. Once consent has been given, further history may be taken to rule out other comorbidities especially related to CK. Then blood will be withdrawn from the patient for the required investigations. For this study 3mls of the patient's blood is required. This will be drawn from the patient at 48hrs after injury.

Confidentiality

The information you provide will be kept confidentially and will only be used for the purpose of this study. At the end of the study, the questionnaires will be destroyed and no personal identifying information will be included in the results.

Cost

You will not be charged for the cost of doing the CPK or BUN at 48hours, but you are expected to cover the rest of the cost required for your treatment, i.e admission fee, medication, xays, other blood tests required, implant cost, theatre fee, nursing fees and doctors fee as charged by the hospital. There will be no exchange of money between the principal investigator, the assisstants and the patient or patient relatives.

Any risks?

The known risks associated with venipuncture include: severe adverse effect documented is loss of consciousness including tonic clonic seizures and injury to anatomical structures in the vicinity. The less severe adverse effects are pain at the site of venipuncture, anxiety, fainting, bruising, and hematoma formation.

Benefits from the study

There are no direct benefits from this study but the information obtained will help better understand the severity of soft tissue injury specifically skeletal muscles associated with fractures of the femur.

Opting out

You are allowed to choose not to participate in this study. This will not compromise your treatment in any way.

CONSENT

I certify that the study has been fully explained to me and I am willing to participate in it.

Participant's Signature (or thumbprint)……

Date……

I confirm that I have clearly explained to the participant the nature of the study and the contents of this consent form in detail and the participant has decided to participate voluntarily without any coercion or undue pressure.

Investigator's Signature…….
Date ……

For Any Enquiries, please contact:

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Appendix IV Ridhaa ya kushiriki katika utafiti huu

Mtafiti mkuu:

Mada ya Utafiti:kiwango cha creatine phospokinase kwenye damu ya wangojwa waliovinjika mifupa ya miguu(femur)

Maelezo zaidi

Utafiti huu unalenga kupima kiwango cha creatine phospokinase kama maka biolojia kwenye damu ya wangojwa waliojeruhiwa na kuvunjika mifupa ya miguu.

Kinachohitajika kutoka kwa mgonjwa au anayepeana maelezo kwa niaba ya mgonjwa:

Mgonjwa atakaposhughulikiwa na kupewa matibabu yanayohitajika ili kumtoa katika hali ya harari,mtafiti mkuu au msaidizi wake ataomba idhini ya mgonjwa ili achukue damu. Damu hii ambayo haitapita mililita tatu (3mls) itapelekwa kwenye maabara ambapo utafiti utafanywa.

Usiri

Matokeo ya utafiti huu yatakuwa siri wala hayatapewa mtu yeyote yule asiyehusika kwenye utafiti huu. Pia maelezo yanayomtambulisha mgonjwa na fomu alizojaza kwa minajili ya utafiti huu, zitaharibiwa ili kudumisha usiri wa utafiti huu.

Malipo

Malipo ya watakao shiriki utafiti huu ni bure wala hawatalipishwa kupimwa kiwacho cha creatine phosphokinase. Hata hivyo ni bora kukumbuka kuwa malipo mengine kama kuonwa na daktari,kulazwa, xray au vifaa viovyote vitakavyohitajika kwa matibabu ya mgojwa havitagaramiwa na utafiti huu baili mgojwa mwenyewe.

Hatari

Hakuna hatari yoyote inayotarajiwa

Faida ya utafiti

Matokeo ya utafiti huu yatasidia kuelewa zaidi kuhusi majeraha ya tishu yanayowakumba waliovunjika mifupa ya miguu

Kujiuzulu kutoka kwa utafiti huu

Kushiriki katika utafiti huu ni kwa kujitolea na ikiwa kwa sababu moja au nyingine hungependa kushiriki,una uhuru wa kujiuzulu. Uamuzi huu hautaadhiri matibabu yako au jinsi utakavyo hudumiwa.

RIDHAA

Nathibitisha kuwa nimesoma na kumweleza mshiriki kuhusu utafiti huu na kulingana na maaelezo yote amekubali kushiriki utafiti huu

Sahihi ya mtafiti..... tarehe.....

Sahihi ya mshiriki.....tarehe.....

Ukiwa na maswali yeyote kuhusu utafiti huu, wasiliana na:

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Appendix V Computer program used to derive the sample size

Borrowed from Bonnet 2011- computer program used to derive the sample size.

```
sizeCIcorr <- function(alpha, corr, CI2w) {
# Computes sample size required to estimate a correlation with desired precision
# Args:
# alpha: alpha level for 1-alpha confidence
# corr: planning value of correlation
# CI2w: desired confidence interval width
# Returns:
# required sample size
z <- qnorm(1 - alpha/2)
n1 <- ceiling(4*(1 - corr^2)^2*(z/CI2w)^2 + 3)
zr <- log((1 + corr)/(1 - corr))/2
se <- sqrt(1/(n1 - 3))
LL0 <- zr - z*se
UL0 <- zr + z*se
LL <- (exp(2*LL0) - 1)/(exp(2*LL0) + 1)
 UL <- (exp(2*UL0) - 1)/(exp(2*UL0) + 1)
N <- ceiling((n1 - 3)*((UL - LL)/CI2w)^2 + 3)
return(N)
}

sizeCIcorr(0.05, 0.95, 0.05)
```

Appendix VIII Summary of the variables recorded table 2

	LAB NO.	AGE IN YRS	SEX	CK	UREA	CREATINE	GFR	UREA: CREATINE RATIO	FRACTURE PATTERN	GRADED MOI	ABI
1	729985298	49	M	3340	19.7	190	39	104	8	HIGH	1
2	729985300	22	M	4843	3.9	79	>89	49	3	HIGH	1
3	729985299	25	M	8267	5.8	79	>89	73	3	HIGH	1
4	729985626	24	M	3713	2	55	>89	36	3	MOD	1
5	729985861	49	M	13453	8	65	>89	123	6	HIGH	0.9
6	729985862	41	M	529	1.6	49	>89	33	3	LOW	1
7	729987362	30	F	1170	2.3	38	>89	61	2	MOD	1.1
8.	729987360	30	F	966	2.9	46	>89	63	2	MOD	1
9.	729987786	24	M	3274	2.3	52	>89	44	5	HIGH	1
10.	729992145	67	M	5149	4.8	83	84	57	8	HIGH	1.1
11	729992144	18	M	5887	2.9	46	>89	46	2	MOD	0.9
12	729992675	52	M	42	2	58	>89	34	2	LOW	1

13	729992816	32	F	3420	5.4	73	>89	73	4	HIGH	1
14	729992817	56	F	4143	2.3	59	>89	38	4	HIGH	1.1
15	729992818	48	M	2306	3.3	74	>89	45	8	HIGH	1
16	729992815	22	M	1049	2.4	49	>89	45	2	LOW	1
17	729993802	29	M	868	2.6	60	>89	43	1	LOW	0.9
18	729993803	35	M	119	4.8	48	>89	10	1	LOW	1
19	729994134	20	M	4060	2.8	65	>89	43	7	HIGH	0.9
20	729994464		M	3682	8.4	99	86	84	1	LOW	1
21	729994840	23	F	792	2.3	64	>89	34	3	LOW	1
22	729994972		M	2185	4	51	>89	78	2	MOD	1.1
23	730738429	53	F	1602	3.5	56	>89	63	3	MOD	1
24	729000606		M	1499	4.5	75	>89	60	6	MOD	1.1
25	729000607		M	2565	4.5	55	>89	81	3	MOD	1.2

26	730738514		F	117	4.2	87	76	48	1	LOW	1
27	729000972	55	F	3311	4	78	>89	51	6	HIGH	1.2
28	729001683	39	M	5943	3.9	73	>89	53	3	HIGH	1.2
29	729002636		M	346	5.5	121	68	45	2	LOW	1
30	729002661		M	4054	5.5	83	>89	66	5	MOD	1
31	729002549	19	F	4002	2.6	49	>89	53	7	HIGH	1..2
32	729002650		M	536	4.4	111	>89	39	3	MOD	1
33	729002823	25	M	1208	3.6	49	>89	73	3	MOD	1
34	729002822	46	M	1254	6.5	62	>89	104	2	MOD	1.1
35	729002824	20	M	7715	4.1	47	>89	87	3	MOD	1.3
36	729003008	18	F	6780	2.6	48	>89	54	3	HIGH	1.4
37	729003016	52	M	1241	2.7	72	>89	38	3	MOD	1
38	729003078	33	M	2423	2.7	62	>89	44	2	MOD	1.2
39	729003777	55	F	189	2.3	52	>89	44	1	LOW	1.2

40	729003976	52	M	1193	3.2	56	>89	57	5	HIGH	0.9
41	729004858	41	M	6276	3.8	69	>89	55	8	HIGH	1.1
42	729012323	18	M	776	2.9	64	>89	45	3	LOW	1
43	729012927		M	238	3.5	28	>89	125	1	LOW	1
44	729012926	37	M	1347	3.1	63	>89	49	6	MOD	1.1
45	729013374	42	M	2601	5	67	>89	74	3	MOD	1.1
46	729013373	28	M	6937	2.7	75	>89	36	3	MOD	1.1
47	729013375	33	M	2889	2.5	90	>89	27	3	MOD	1.1
48	729013424		F	57	5	56	>89	89	1	LOW	1
49	729013425	38	F	864	3.6	73	>89	49	1	LOW	1
50	729013481	30	M	1950	2.3	60	>89	38	1	LOW	1.2
51	729013551	36	M	1942	2.8	57	>89	49	3	MOD	1
52	729013786	25	M	8623	4	52	>89	77	3	HIGH	0.9

53	729014300	32	M	3998	5.6	138	58	40	4	HIGH	1
54	729014299	54	F	952	4.1	82	70	50	1	MOD	0.9
55	729014298	38	M	892	3.8	71	>89	54	3	MOD	0.9
56	729014297	25	M	7801	3.5	55	>89	64	9	HIGH	1
57	729014294	23	M	812	2.3	37	>89	62	1	LOW	1
58	729014295	27	M	810	2.4	53	>89	45	1	LOW	1
59	729014296	30	M	11000	4.1	79	>89	52	1	HIGH	1
60	729014762	65	F	170	3.6	58	>89	62	4	LOW	1
61	729014767	19	M	1345	2.9	58	>89	50	3	MOD	1
62	729014772	40	M	1027	6.4	62	>89	103	6	MOD	1.1
63	729014779	37	M	3282	6.2	92	>89	67	2	MOD	0.9
64	729014968	31	M	1540	3.6	75	>89	48	3	MOD	1
65	729015157	25	M	4185	6.2	98	>89	63	3	HIGH	1.1
66	729015385	31	M	4441	4.8	78	>89	78	6	HIGH	0.9

Appendix IX Budget

ITEM	QUANTITY	UNIT PRICE	TOTAL
Operating Costs:			
Internet: Safaricom	12GB	-	3000/-
Stationary:			
Pens (Box)	1	400/-	400/-
Writing pads	5	200/-	1000/-
Printing paper (rim)	1	1200/-	1200/-
Printing Cartridges	4	1200/-	4800/-
Binding Fees	5	100/-	500/-
Approval:			
Ethical Review Fee	1	2000/-	2000/-
Consultation:			
Statistician	1	4000/-	4000/-
Patient Cost	-	-	-
Cost of CK	66	399/-	26,334/-
BUN	66	550/-	36,300/-
TOTAL			79,534/-

Appendix X Other certificates relevant to the study