CORRELATION BETWEEN PEAK TRANSFORMING GROWTH FACTOR BETA 1 PLASMA LEVELS AND EARLY STAGE OF RADIOLOGICAL UNION IN PAEDIATRIC SUPRACONDYLAR HUMERUS FRACTURES AT KENYATTA NATIONAL HOSPITAL

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Declaration

Declaration

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Dedication

This book is dedicated to my family for their unwavering support, patience and inspiration.

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List of Abbreviations

AAOS	American Academy of Orthopaedic Surgeons
AIS	Abbreviated Injury Scale
CRPP	Closed Reduction and Percutaneous Pinning
ELISA	Enzyme Linked Immunosorbent Assay
KAVI	Kenya AIDS Vaccine Initiative
KNH	Kenyatta National Hospital
KNH-UON ERC	Kenyatta National Hospital – University of Nairobi Ethics and Research Committee
LAP	Latency Associated Peptide
LTBP	Latent TGFB1 Binding Protein
MISS	Modified Injury Severity Score
MMPS	Matrix Metalloproteinases
ORIF	Open Reduction and Internal Fixation
PDGF	Platelet Derived Growth Factor
SCHF	Supracondylar Humerus Fractures
SPSS	Statistical Package for Social Sciences
TBI	Traumatic Brain Injury
TGFB	Transforming Growth Factor Beta
TGFB 1	Transforming Growth Factor Beta 1
UON	University of Nairobi

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Abstract

Study Background: Transforming growth factor beta 1 is currently only being evaluated for experimental rather than diagnostic or therapeutic purposes. It plays multiple roles in tumour biology as well as wound and fracture healing. Supracondylar humerus fractures are common paediatric injuries classified and managed according to the Wilkin's modification of the Gartland's classification. The purpose of this study was to determine the association between peak plasma level of activeTGFB1 and the stage of radiological union 3 weeks post injury or surgery in patients with paediatric supracondylar humerus fractures.

Broad Objective: To determine the association between peak active TGFB 1 plasma level and the stage of radiological union in patients with paediatric supracondylar humerus fractures 3 weeks after injury or surgery.

Hypothesis: A higher peak plasma level of TGFB1 does not affect the odds of having a more advanced stage of radiological union in paediatric supracondylar humerus fractures**.**

Materials and Methods: The study was carried out between December 2021 and March 2022 at the Kenyatta National Hospital. An analytical cross sectional study design was employed. 44 patients with paediatric SCHF were recruited using simple random sampling. TGFB1 plasma level was assayed via ELISA while the stage of radiological fracture union evaluated using a pre-designed ordinal scale 21 days after injury or surgery.

Statistical Analysis: Descriptive data was analysed using the SPSS© version 24 and presented as means and percentages. Independent samples t test was used to compare means. Ordinal logistic regression was used for hypothesis testing.

Results: A total of 44 participants were included in the study. The mean age was 6.8 (s.d. = 2.205) years. The proportion of males to females was 72.72% to 27.27%. Mean peak TGFB1 plasma level was 116.28ng/ml (s.d. = 27.23) with no statistically significant difference between levels in males and females (p = 0.878). There was a

significant negative correlation between age and TGFB1 plasma level (r = -0.824) . The odds ratio of having an advanced stage of radiological union with an increased peak plasma level of TGFB1 was 1(p = 0.0869) thus we failed to reject the null hypothesis.

Conclusion: An increase in peak active TGFBI plasma level (ng/ml) does not affect the odds of having an advanced stage of radiological union at 3 weeks post injury or surgery in paediatric supracondylar humerus fractures.

CHAPTER 1: INTRODUCTION

1.1 Background

TGFB1 is expressed in all body tissues and cells, is released in response to injury and plays a pivotal role in tumour biology as well as in fracture and soft tissue healing (1). Supracondylar humerus fractures (SCHF) are common paediatric injuries mostly resulting from falls on the outstretched hand. They are classified and managed using the Wilkins modification of Gartland's classification.

Transforming growth factor beta is currently only being evaluated for experimental rather than diagnostic or therapeutic purposes. It plays a pivotal role in tumorigensis as well as wound and fracture healing. Rapid healing of fractures among patients with traumatic brain injury (TBI) has been hypothesized to be as a result of increased production of this growth factor in conjunction with other cytokines (2). However, the exact mechanisms are yet to be fully understood (2). Currently, there are no studies correlating TGFB1 levels and the rates of healing in paediatric fractures. Additionally we do not have local reference range values for this growth factor in normal/healthy subjects.

This purpose of this study was to determine the association between peak TGFB1 plasma levels and the stage of radiological union 3 weeks after injury or surgery in patients with paediatric SCHF at Kenyatta National Hospital.

CHAPTER 2: LITERATURE REVIEW

2.1 Transforming Growth Factor Beta 1

2.1.1 Overview

Transforming Growth Factor Beta 1 (TGFB1) is one of the TGFB isoforms (3). It is a cytokine with multiple functions that belongs to the superfamily of transforming growth factors. TGFB1 plays important roles in fracture and wound healing, angiogenesis, apoptosis control, immune regulation and tumour biology (3). All cells have receptors for TGFB1 and it is mainly produced by immune system cells and platelets. Forty per cent of all TGFB1 found in peripheral blood plasma is secreted by platelets. TGFB1 is initially produced in its latent form and subsequently undergoes activation via different mechanisms (3). In vivo half-life of active TGFB1 is 2-3 minutes and up to 83% is excreted through biliary secretion (3). There is no available local data on either normal reference range values or pre/post injury levels for plasma or serum TGFB1.

2.1.2 Platelet TGFB1

TGFB1 is present in two separate pools stored within alpha granules of platelets. The first pool, representing 95 per cent of total TGFB1 in platelets, is complexed with a Latent TGFB1 Binding Protein (LTBP) and a Latency Associated Peptide (LAP) (3) (4). The second pool is a complex of TGFB1 and LAP without LTBP. These two pools are secreted into plasma in two distinct ways. The first pool of TGFB1+LTBP+LAP is released briefly during the process of blood clotting. The second pool of TGFB1+LAP is 'trapped' in the clot before being released into bloodstream by further downstream activation (3) (4). The significance of this double mode secretion of TGFB1 in physiological as well as pathological processes remains unexplored. Most organs in healthy individuals contain more latent TGFB1 than would be required to cause tissue fibrosis. Therefore, regulation of TGFB1 in mitigating fibrotic disease depends on its activation rather than its synthesis or secretion (3) (4).

2.1.3 Activation

TGFB1 secreted by platelets in its latent form is activated shortly thereafter (3). This process is dependent on the presence of various factors secreted from platelet granules together with TGFB1. The nature of these substances is still not well understood but some of the ones identified include thrombin and collagen (3) (4). These molecules activate latent TGFB1 differently: while thrombin causes a short lived cytokine 'burst', collagen stimulates a prolonged TGFB1 leakage from platelets. (3) (4)

2.1.4 Signalling

TGFB1 signalling is by either the canonical or non-canonical pathway. All three isoforms of TGFB utilise a similar receptor that contains the following components: RI, RII and RIII. RIII binds TGFB1 and recruits it to RII. This leads to phosphorylation of R1 resulting in the formation of a serine/threonine kinase complex (3) (4).

This complex induces the C-terminal phosphorylation of certain homologues of the Drosophila protein referred to as SMADs (3) (4). These SMADs form complexes with co-mediators which are subsequently trans located to the cell nucleus where they regulate the transcription of numerous genes. This is referred to as the canonical pathway of TGFB1 signalling. Non- canonical signalling on the other hand involves activation of other pathways including MAPK and Rho GTPase pathways (3) (4).

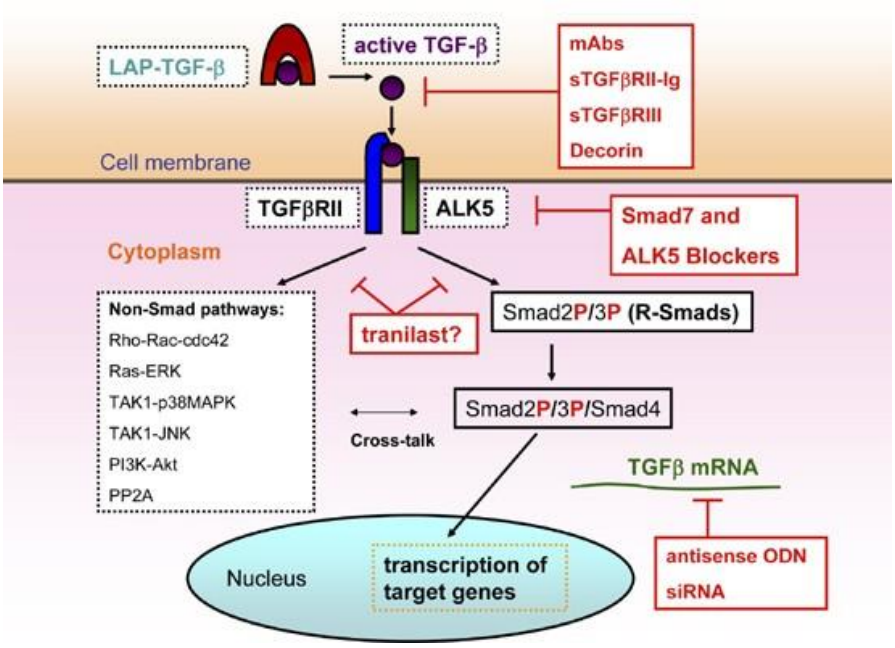

Figure 1: TGFB1 signalling pathways (3)

2.2 Paediatric Trauma

2.2.1 Overview

Trauma contributes significantly to morbidity and mortality among infants in developed countries (5). Among paediatric patients sustaining severe trauma, skeletal injuries are present in 10 – 15 per cent. Blunt trauma contributes to 80 per cent of injuries (5). Injury mechanisms vary among different age groups. Non- accidental trauma and falls are common in infants and toddlers. On the other hand motor vehicle collisions and sports related injuries predominate among older children and adolescents (5).

2.2.2 Modified Injury Severity Scale

The Modified Injury Severity Scale (MISS) is a simplified adaptation of the Injury Severity Score used in paediatric trauma (6). It involves scoring injuries to five key body regions according to the Abbreviated Injury Scale (AIS) and obtaining the sum of the squares of the three body regions with the highest scores (6). Its utility is in the prediction of morbidity and mortality in paediatric trauma (6)

2.3 Paediatric Supracondylar Humerus Fractures

2.3.1 Epidemiology

Paediatric SCHF are the most common elbow injuries in this age group accounting to up to 15 per cent (7). Most SCHF are of the extension type. They commonly occur as a result of falling onto the outstretched hand (8). Boys account for more cases than girls and the non-dominant limb is affected more than the dominant one. Majority of these injuries tend to be closed injuries (8).

2.3.2 Classification

Paediatric SCHF are classified according to the Wilkins' modification of Gartland's original classification (9) (10).

Table 1: Modified Gartland's Classification (10).

Classification.	**Description**
Type I	Non- displaced
Type II	Anterior displacement with an intact posterior hinge
Type IIA	No rotational instability
Type IIB	Rotational Instability
Type III	Displaced with no cortical contact
Type IV	Disruption of posterior periosteum – intra-operative finding

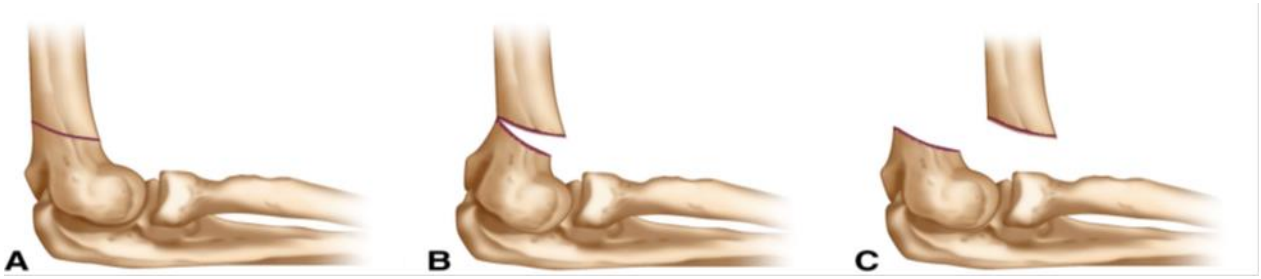

Figure 2: Modified Gartland's Classification (10).

2.3.3 Management

Current management of paediatric SCHF are based on the modified Gartland's classification as recommended by the American Academy of Orthopaedic Surgeons (AAOS) (11). Type I and IIA fractures are treated with closed reduction and casting. Type IIB, III and some type IIA injuries that fail non-operative treatment are managed by either closed reduction and percutaneous pinning (CRPP) or open reduction and Kirschner wiring (K- wiring) (9) (11).

2.3.4 Complications

Early complications of paediatric SHF include injury to neurovascular structures as well as compartment syndrome. Elbow stiffness, cubitus varus/valgus, pin track infection, Volkman ischaemic contracture and myositis ossificans are some of the medium to long term complications (12).

2.4 Fracture Healing

2.4.1 Overview

The process of fracture healing follows a specific time sequence involving interdependent cellular as well as molecular events (13).

2.4.2 Stages

Healing of fractures involves responses from cortical bone, periosteum, bone marrow as well as inflammatory mediators and cells (13). Fracture stability determines the type of healing that will occur in fractures (13). Stabilizing a fracture with rigid internal fixation where the strain is less than 2 per cent results in primary cortical healing that involves harvesian remodelling by osteoclasts and osteoblasts. Rigid internal fixation is mainly achieved when fractures are fixed with compression plating (13).

In the presence of non-rigid fracture fixation, micro motion is present at the fracture site resulting in secondary bone healing (13). Non-rigid fixation is achieved when fractures are fixed with intra-medullary nailing, bridge plating, external fixation or application of a cast (13). The process of secondary bone healing progresses in three main phases.

i. Inflammation

Haematoma formation occurs within the first 24 hours of a fracture and provides a source of haematopoietic cells that secrete various cytokines and growth factors (13). Macrophages, neutrophils and platelets produce cellular mediators including TGFB, interleukins 1, 6 and 10; tumour necrosis factor alpha as well as platelet derived growth factor (13).

ii. Repair

Cytokines stimulate differentiation of mesenchymal cells from the periosteum to form chondrocytes that secrete extracellular matrix forming a hyaline cartilaginous model at the fracture site (13). This process of chondrogenesis occurs within the first 7 to 10 days. Fibroblasts also migrate to the fracture site leading to formation of granulation tissue. By day 14, primary callus has been formed (13).

At 4 to 5 weeks, chondrocytes at the centre of the cartilage model undergo hypertrophy and start producing collagen type 10 and fibronectin in their extracellular matrix. This altered matrix promotes cartilage calcification (13). Voids created within the cartilage model allow blood vessels to invade to deliver stem cells that transform into bone forming osteoblasts that produce osteoid to form hard callus or woven bone (13).

iii. Remodelling

Cartilage calcification during woven bone formation prevents nutrients from reaching the chondrocytes and results in them undergoing apoptosis. Chondroclasts also play a role in the degradation of this calcified cartilage (13). Osteoclasts also invade and aid in converting woven bone into a structure that resembles normal bone, a process called remodelling (13).

2.4.3 Diaphyseal Versus Metaphyseal Fracture Healing

Diaphyseal and metaphyseal bone heal following fundamentally similar principles (13).Whether a metaphyseal fracture heals via intramembranous bone formation or

via a combination with endochondral bone formation is dependent on the inter fragmentary strain across the fracture site. Inter fragmentary strain of less than 2 per cent favours primary bone healing via the intramembranous route leading to minimal callus formation (13).

2.4.4 Paediatric Fracture Healing

Paediatric fracture healing is similar to adult bone healing (14). They both undergo the phases of inflammation, repair and remodelling (14). However, the greater sub periosteal haematoma as well as a stronger periosteum in children contribute to the rapid formation of clinically stable callus when compared to adults (14). Genes and hormones responsible for skeletal development and fracture healing are similar. This osteogenic environment of the paediatric bone means that the healing process is already in progress at the time of injury (14). These factors contribute to rapid healing of paediatric fractures in comparison to adult fractures (14).

2.4.5 Radiological Assessment of Paediatric Fracture Healing

A study by Prosser et al in 2011 reviewed two hundred and twenty eight radiographs of paediatric patients younger than 6 years of age in an attempt at establishing which radiological features can accurately date healing paediatric fractures (15). The results of their study are summarised in the table below.

Table 2: Timetable for Paediatric Fracture Healing (15).

Radiological Feature	First Seen* (Days)	Peak Period* (Days)	Last Seen* (Days)
Soft tissue swelling	1	1 – 2	31
Periosteal reaction	5	15 – 35	96
Soft Callus	12	22 – 35	66
Hard Callus	19	>22	96
Bridging	19	>36	300
Remodelling	45	>36	421

*From date of injury

Islam et al. in 2000 attempted to establish a timetable for expected radiographic changes visible during bone healing in children (16). Their study included 707 radiographs of 141 patients who had diaphyseal, dia-metaphyseal as well as metaphyseal fractures of the forearm (16). They evaluated for the timelines for appearance of eight radiographic features of fracture healing and compared them against expected timelines in histologic stages of fracture healing. Their findings are summarized in the table below.

Table 3: Timetable for Radiographic Features of Fracture Healing (16).

Histologic Stages of Fracture Healing	Study Feature	Week of Onset After Injury	Peak (Week)
Inflammation (Week 0-3)	Fracture gap widening	3	4-6
Soft callus (Week 2-6)	Sclerotic fracture margin	3	4-6
	Periosteal reaction	2	4-7
	Callus presence	2	4-7
Hard callus (Week 2-13)	Increased callus density > cortex	5	13
	Bridging	5	13
	Periosteal reaction incorporation	7	14
Remodelling (Week 12-104)	Remodelling	4	9

2.4.6 Bone Healing After Traumatic Brain Injury

In the last 30 years scientific evidence has linked the rapid and robust callus formation in polytrauma patients to the presence of traumatic brain injury (2). Various cytokines and growth factors, including TGFB1, have been studied in an

attempt to explain this phenomenon (2). However, the mechanism responsible for this phenomenon is not well understood (2).

2.5 TGFB1 Levels in Fracture Healing

TGFB1 is expressed both locally at a fracture site and distributed systemically leading to increased plasma levels in circulating blood (17) (18). Within 24 hours of a fracture, during the inflammatory phase of fracture healing, TGFB1 is presented within the forming haematoma, its main source being alpha granules of platelets (17) (18). Other cells of the immune system such as monocytes, macrophages as well as T cells also synthesize TGFB1 (18). Several days later the reparative phase is initiated via two stages that overlap. These are intramembranous ossification phase and the endochondral ossification phase (18) (1). TGFB1 expression is most pronounced during this phase within the forming callus and surrounding cells that include osteoblasts, osteocytes, chondroblasts and chondrocytes (1).

Various authors in available literature have reached concurrence in the conclusion that TGFB1 levels reach peak levels between day 14 and 21 after a fracture followed by a gradual decline from week 3 to 24 (19) (20) (21). Significant differences have also been observed in patients with physiological bone healing versus those with delayed or non-union (19). One cause that has been correlated with decreased TGFB1 levels and delayed union in adult long bone fractures is cigarette smoking (22).

2.6 Soft Tissue Injury in Trauma

2.6.1 Overview

Soft tissue injuries, similar to organ injury and fractures, form the first hit after multiple injury (23). Host defence responses are generated with release of various molecular mediators in an effort to promote healing (23). TGFB1 plays a pivotal role in soft tissue healing after trauma (23).

2.6.2 Phases of Soft Tissue Healing

Haemorrhage and tissue damage after blunt trauma precipitates microvascular and cellular events that progress in four phases.

2.6.2.1 Inflammatory Phase

Trauma leads to soft tissue destruction and disrupts the micro-circulation in the injured tissue (24). Exposed sub endothelial collagen causes activation and aggregation of platelets as well as activation of the coagulation and complement cascades in an attempt to stop the bleeding (24). Inflammatory mediators including kallikrein, prostaglandins as well as histamine cause increased endothelial permeability resulting in edema and worsening tissue hypoxia (25) (24).

Release of multiple cytokines initiates a localized inflammatory response. Platelet derived growth factor (PDGF), TGFB1, serotonin, epinephrine and Thromboxane A2 cause chemotaxis of macrophages, neutrophils, fibroblasts and lymphocytes. Granulocytes, including neutrophils and macrophages migrate to the area of tissue destruction to provide the initial defence against bacteria and initiate wound debridement. Macrophages further the inflammatory process by releasing additional cytokines. (25) (24) (26).

2.6.2.2 Proliferative Phase

Fibroblasts migrate to the site of injury, proliferate and produce collagen. Endothelial ingrowth into the new extracellular matrix occurs and results in angiogenesis. The concentration of capillary beds as well as water is increased during this phase in comparison to normal tissue. This phase peaks in the second week after tissue injury (25) (24) (26).

2.6.2.3 Reparative Phase

During this phase, collagen cross linking occurs as water content and vascularity of the tissue decline (25) (24).

2.6.2.4 Remodelling Phase

This phase lasts until 6 to 24 months after initial injury. Vascular regression, granulation tissue remodelling as well as production of new ECM proteins occurs. Type III collagen is replaced by type I collagen. Production of these new proteins is promoted in part by TGFB1 and platelet derived growth factor (PDGF). TGFB1 inhibits expression of MMPSs leading to laying down of more collagen. EGF produced by platelets and macrophages increases production of MMPSs by fibroblasts during the remodelling phase (25) (27).

2.7 Conceptual Framework

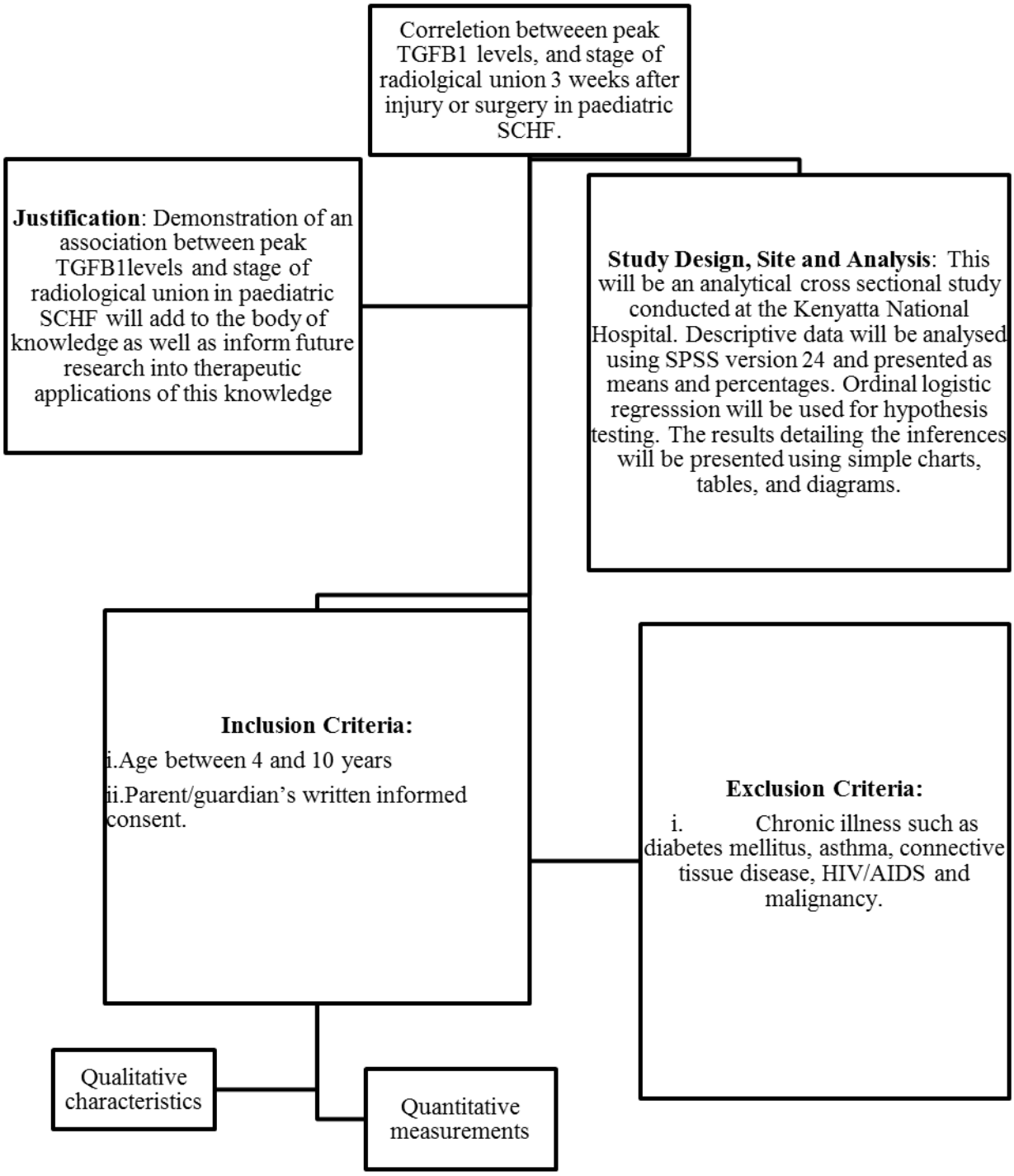

Figure 3: Conceptual Framework

2.8 Study Justification

Demonstration of an association between peak TGFB1 plasma levels and the stage of radiological union at 3 weeks post injury or surgery in patients with paediatric supracondylar humerus fractures will provide additional knowledge on the role of TGFB1 in fracture healing and inform future research on its clinical and therapeutic application in management of delayed as well as non-union of these fractures.

2.9 Hypothesis

A higher peak plasma level of TGFB1 does not affect the odds of having a more advanced stage of radiological union in paediatric supracondylar humerus fractures.

2.10 Study Objectives

2.10.1 General Objective

To determine the association between peak TGFB1 plasma levels and the stage of radiological union 3 weeks after injury or surgery in patients with paediatric SCHF.

2.10.2 Specific Objectives

i. To measure peak active TGFB1 plasma levels 3 weeks after injury or surgery in patients with paediatric SCHF.

ii. To determine the stage of radiological union 3 weeks after injury or surgery in patients with paediatric SCHF based on a pre-designed ordinal scale.

iii. To determine whether there is an association between peak TGFB1 plasma levels and the stage of radiological union 3 weeks after injury or surgery in patients with paediatric SCHF.

CHAPTER 3: MATERIALS AND METHODS

3.1 Study Setting

The study was conducted at the Kenyatta National Hospital paediatric orthopaedic ward, the orthopaedic outpatient clinic and the accident and emergency department. Kenyatta National Hospital is a level six national teaching and referral hospital located in Nairobi, Kenya.

3.2 Research Design

An analytical cross sectional study design was employed.

3.3 Target Population

This study involved all paediatric SCHF patients presenting to KNH who met the inclusion criteria. Participation was purely on voluntary basis.

3.4 Sampling procedure

Simple random sampling method was used on all patients with paediatric SCHF until a desired sample size of 44 was attained. The patients were taken through an overview of the study before going through the eligibility criteria to determine their eligibility for the study. Those who met the criteria were recruited.

3.5 Inclusion and Exclusion Criteria

3.5.1 Inclusion Criteria

i. Age 4 – 10 years.
ii. Parent/guardian's consent.
iii. Acute extension type SCHF.

3.5.2 Exclusion Criteria

i. Chronic illness such as diabetes mellitus, asthma, connective tissue disease, HIV/AIDS and malignancy.
ii. Flexion type SCHF.
iii. Patients younger than 4 or older than 10 years of age.
iv. Malunited SCHF.

3.6 Study Procedure

3.6.1 Ethical Considerations

Permits - Approval for the study was obtained from the Kenyatta National Hospital – University of Nairobi Ethics and Research Committee (KNH-UON ERC), a copy of which is attached herein.

Principles - The study was undertaken while observing the Declaration of Helsinki on use of human subjects.

Consent - Verbal explanation of the objective of the study was provided and written informed consent obtained from parents or legal guardians of all the study participants.

COVID – 19 prevention measures were strictly observed.

3.6.2 Data Collection Procedures

3.6.2.1 Demographic and Clinical Data

Data was collected detailing the patients age, gender, type of injury based on Gartland's classification, type of treatment (operative versus non- operative) and the type of operative treatment (CRPP versus ORPP).

3.6.2.2 Peak Active TGFB1 plasma level

a) Sample collection

10 millilitres of peripheral whole blood was drawn from a vein in the ante-cubical fossa of the uninjured upper limb of each participant 3 weeks after injury or surgery. The blood sample was collected in a sodium heparin vacationer and transported to the lab within 24 hours.

b) Sample Processing

The blood sample was then centrifuged to separate plasma from the cellular components. Plasma was frozen at -20 degrees Celsius awaiting assay.

c) Assay

TGFB1 assays were performed at the Africa Biosystems Laboratories in Nairobi. A sandwich Enzyme Linked Immunosorbent Assay (ELISA) technique was employed using the Invitrogen Human TGF beta1 ELISA Kit manufactured by Thermo Fisher Scientific Inc. © The values were recorded as numerical values in nanograms per millilitre.

3.6.2.3 Radiological Fracture Union

This was determined by assessment of digital radiographs of the affected limb taken 3 weeks after injury or surgery by two consultant radiologists. The findings were defined and recorded on a pre-designed ordinal scale as follows:

1. Soft tissue swelling – disruption of soft tissue planes.
2. Periosteal reaction – Presence of elevation in a linear fashion and areas of calcification of the periosteal sleeve next to the fracture site.
3. Soft callus – New bone formation at the fracture site that is fluffy in appearance.
4. Hard callus – New bone formation that nearly resembles normal cortex in its density and is well demarcated.
5. Bridging – Obliteration of fracture line with fracture gap bridging.
6. Remodelling – Conversion of woven bone into a lamellar pattern that resembles that of original bone.

3.7 Statistical Analysis

Data was collected and entered into SPSS© version 24. Descriptive data was analysed and presented as means on tables, pie charts and bar graphs. Independent Samples t Test was used to investigate for significant differences between means while ordinal logistic regression was utilised to evaluate the association between active TGFB1 plasma level and the stage of radiological union at 3 weeks post injury or surgery in children with supracondylar humerus fractures.

CHAPTER 4: RESULTS

4.1Introduction

A total of 44 patients with paediatric supracondylar humerus fractures were recruited into the study.

4.1.1 Age, Gender and Injury Type Distribution

4.1.1.1 Age

Table 4: Age Distribution

	N	Minimum	Maximum	Mean	Std. Deviation
Age	44	4	10	6.8	2.205

The mean age of participants in the study was 6.8 years (s.d. = 2.205)

4.1.1.2 Gender

Table 5: Gender Distribution

Male	32	72.72%
Female	12	27.27%
Totals	44	100%

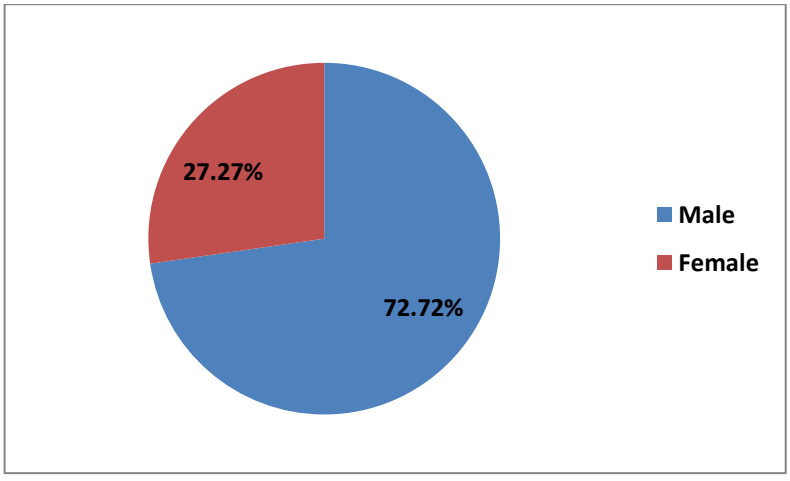

Figure 4: Gender Distribution

Majority of the participants were males at 72.72% while females represented 27.27%.

4.1.1.3 Injury Type

Table 6: Distribution of Injuries Based on the Modified Gartland's Classification

Type	Number
Type I	14
Type IIA	7
Type IIB	6
Type III	17

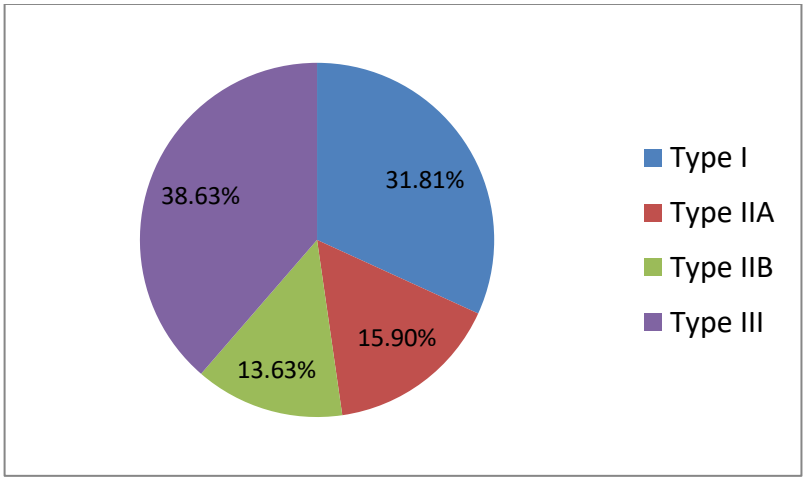

Figure 5: Distribution of Injuries Based on the Modified Gartland's Classification

Based on the Modified Gartland's classification majority of the participants (38.63%) had type I injuries. 31.81%, 15.90% and 13.63% had types IIA,IIB and III respectively.

4.1.2 Peak Active TGFBI Plasma Level

Table 7: Mean Active TGFB1 Plasma Level

	N	Minimum	Maximum	Mean	Std. Deviation
TGFBI (ng/ml)	44	77.26	189.12	116.28	27.23

The mean active TGFB1peak plasma level was 116.28ng/ml (s.d. = 27.23ng/ml)

4.1.2.1 Age Versus Active TGFB1 Plasma Level

Table 8: Age Plotted Against Active TGFB1 Level

Age (Years)	TGFB1 Level (ng/ml)	Age (Years)	TGFB1 Level (ng/ml)
10	91.7	10	82.55
10	92.61	5	131.01
4	189.12	9	91.24
4	153.67	4	158.47
4	147.05	10	86.66
8	110.01	8	100.86
5	129.19	4	121.22
4	180.41	8	114.11
7	117.32	5	95.57
10	93.55	6	112.74
4	136.53	10	93.52
8	94.43	6	120.98
6	130.58	7	77.26
5	129.22	4	139.72
9	95.37	7	104
8	96.62	5	135.61
10	96.23	9	90.79
6	115.93	6	111.36
4	183.14	4	134.7
7	109.98	8	96.61
9	92.34	6	124.18
6	118.22	10	89.88

Table 9: Correlation Between Age and Active TGFB1 Level

Correlation

		AGE	TGFB1
AGE	Pearson Correlation	1	-.824**
	Sig. (2-tailed)		.000
	N	44	44
TGFB1	Pearson Correlation	-.824**	1

	Sig. (2-tailed)	.000	
	N	44	44

**. Correlation is significant at the 0.05 level (2-tailed).

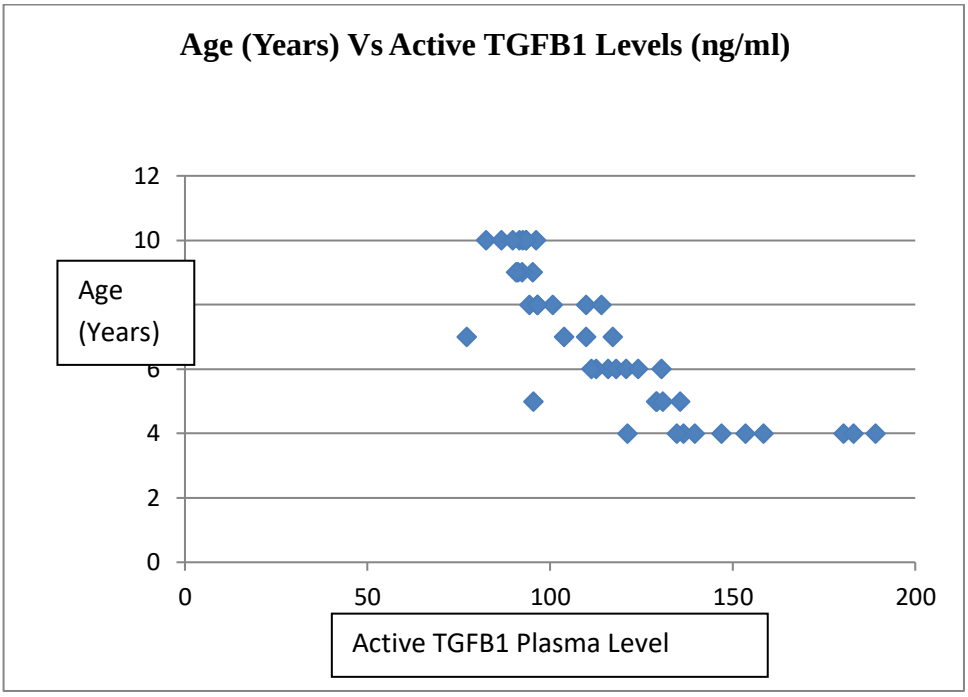

Figure 6: Age Plotted Against Active TGFB1 Level

There was a statistically significant negative correlation between age and active TGFB1 peak plasma level. ($r = -0.824$, $p<0.05$).

4.1.2.2 Gender Versus Active TGFB1 Plasma Level.

Table 10: Mean Active TGFB1 Level Plotted Against Gender

Gender	Mean Active TGFB1 Level (ng/ml)
Male	116.49
Female	115.69

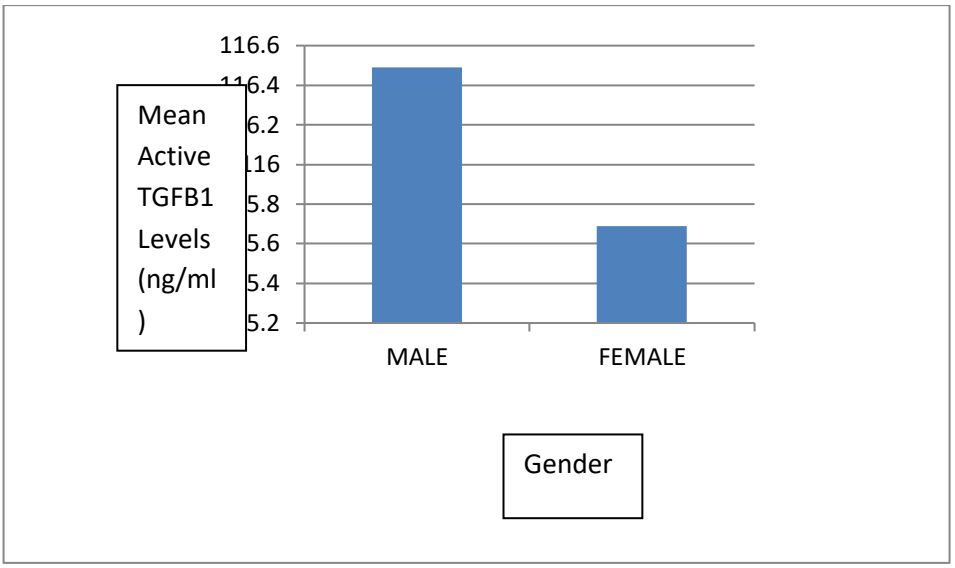

Figure 7: Mean Active TGFB1 Level Plotted Against Gender

Table 11: Comparison Between Mean TGFBI Level in Male and Female Participants

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
Male	32	116.4984	29.53222	5.22061

One-Sample Test

	Test Value = 115.69					
	t	Df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Male Vs. Female	.155	31	.878	.80844	-9.8391	11.4559

There was no statistically significant difference in peak active TGFB1 plasma levels between male and female participants (p = 0.878).

4.1.2.3 Treatment Type Versus Active TGFB1 Plasma Levels

Table 12: Mean Active TGFB1 Level Plotted Against Treatment Type

Treatment Type	Mean Active TGFB1 Level (ng/ml)
Non Operative	111.74
Operative	109.06

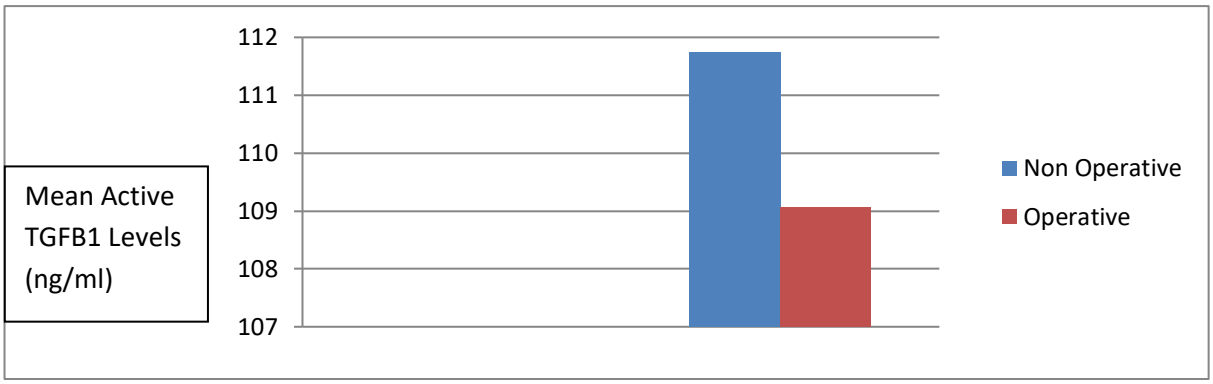

Figure 8: Mean Active TGFB1 Level Plotted Against Treatment Type

Table 13: Comparison Between Mean TGFBI Level in Non - Operative and Operative Groups

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
Operative	30	109.0620	22.50531	4.10889

One-Sample Test

	Test Value = 111.74					
	T	Df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Operative Vs. Non Operative	-.652	29	.520	-2.67800	-11.0816	5.7256

The difference in active TGFB1 plasma level between those who received operative treatment versus those who received non-operative treatment was not statistically significant (p = 0.520).

4.1.2.4 Type of Operative Treatment Versus Active TGFB1 Plasma Level

Table 14: Mean Active TGFB1 Level Plotted Against Type of Operative Treatment

Type of Operative Treatment	**Mean Active TGFB1 Level**
CRPP	109.49
ORPP	108.31

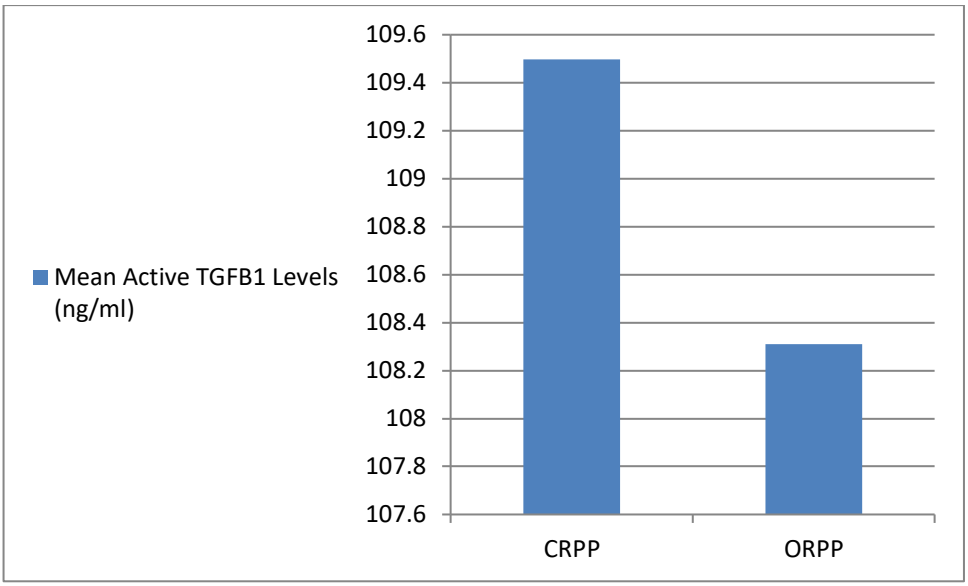

Figure 9: Mean Active TGFB1 Level Plotted Against Type of Operative Treatment

Table 15: Comparison Between Mean TGFBI Level in CRPP and ORPP Groups

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
CRPP	14	107.7643	26.64540	7.12128

One-Sample Test

	Test Value = 108.31					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
CRPP Vs ORPP	-.077	13	.940	-.54571	-15.9303	14.8389

There was no statistically significant difference in active TGFB1 plasma level between CRPP and ORPP groups (p=0.940).

4.1.3 Relationship Between Peak Active TGFB1 Plasma Level and Stage of Radiological Union

Table 16: Ordinal Logistic Regression Analysis Comparing Peak Active TGFB1 Plasma Level and Stage of Radiological Union.

Parameter Estimates

		Estimate	Std. Error	Wald	df	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
Threshold	[UNION = 2]	.632	1.470	.185	1	.667	-2.249	3.513
	[UNION = 3]	3.525	1.749	4.063	1	.044	.098	6.952
Location	TGFB1	-.002	.012	.027	1	.869	-.026	.022

Link function: Logit.

Exp_B	Lower	Upper
1.881	.106	33.546
33.952	.102	1045.588
.998	.974	1.002

CHAPTER 5: DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

The diagnostic application of TGFB1 plasma level depends critically on the control value. However, reliable information on the plasma TGFB1 level of healthy children has not been available, especially within the African population. Peak level of this cytokine after injury has been documented to occur at day 14 to day 21 (17) (19).

We thus measured the peak plasma level of active TGFB1 in a total of 44 children with supracondylar humerus fractures 21 days after injury or surgery depending on the severity of injury using a TGFB1 specific ELISA. Means of these levels between male versus female, operative versus non-operative as well as CRPP versus ORPP groups were compared. Peak TGFB1 plasma level was also compared to the stage of radiological union based on a pre-designed ordinal scale.

The entire age (4-10 years old) related profile of plasma TGFB1 is shown in Table 9. The result showed a significant negative correlation between age and plasma TGFB1 level ($r = -0.824$, $p < 0.05$, $n = 44$. Mean TGFBI plasma value was 116.28ng/ml +/- 27.23ng/ml ($n = 44$). There was no significant difference in the level between males and females (male; 116.49 +/- 29.53 ng/ml, $n = 32$, female 115.69 +/- 28.21 ng/ml, $n = 12$). Similarly no significant differences were noted between operative vs. non operative ($p = 0.520$) and CRPP vs. ORPP ($p = 0.940$) groups (95%CI).

Okamoto et al. showed a negative correlation between age and TGFB1 level in a study involving healthy Japanese individuals of varying ages (28). However, he did not report any significant differences in the level between males and females. Rosenweig et al. also came to a similar conclusion on the relationship between age and TGFB1 level but did not conduct a sex comparison (29). No information on differences of TGFB1 level between surgically versus non-surgically treated individuals with similar injuries has been reported elsewhere, neither is there literature comparing those who have undergone open versus percutaneous techniques. This study showed no statistically significant difference between these two groups ($p=0.940$).

Sarahrudi et al. reported higher TGFB1 level during the early healing period in individuals with physiological bone healing compared to controls but no significant difference between those with physiological and impaired fracture healing. This study, however, did not include children.

In our data analysis, the odds of having an advanced stage of radiological union with an increased active TGFBI plasma level (ng/ml) was 1 (95% CI, 0.974 – 1.022), p = 0.869. Consequently we failed to reject the null hypothesis.

5.2 Conclusion

A high peak plasma level of active TGFB1 does not affect the odds of having a more advanced stage of radiological union 3 weeks after injury or surgery in patients with paediatric supracondylar humerus fractures.

5.3 Recommendations

A larger cohort or cross sectional study should be conducted to further interrogate the utility of TGFB1 in fracture healing. This should include comparison with healthy subjects covering a wider age range and involve serial measurements throughout the entire fracture healing process.

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CHAPTER 6: APPENDICES

6.1 Data Collection Sheet

Unique Study Number: ……..

Date of injury or surgery……...

Date of data collection …….

PART A: DEMOGRAPHIC AND CLINICAL DATA

Variable	Unit of Measurement	Measurement
Age	Years	

Variable		
Sex(TICK APPROPRIATELY)	1. Male ( )	2. Female ( )

	TYPE	TICK APPROPRIATELY
MODIFIED GARTLAND CLASSIFICATION	I	1.
	IIA	2.
	IIB	3.
	III	4.
	IV	5.

		TICK APPROPRIATELY
TREATMENT	OPERATIVE	1.
	NON- OPERATIVE	2.

TYPE OF OPERATIVE TREATMENT		TICK APPROPRIATELY
	CRPP	1.
	OPEN REDUCTION AND K WIRING	2.

PART B: PEAK ACTIVE TRANSFORMING GROWTH FACTOR BETA 1 PLASMA LEVELS

Variable	Unit of Measurement	Measurement
Active TGFB1 Plasma Level	Nano grams / millilitre (ng/ml)	

PART C: STAGE OF RADIOLOGIC UNION AT 3 WEEKS

RADIOLOGIC UNION STAGE :	1. SOFT TISSUE SWELLING 2. PERIOSTEAL REACTION 3. SOFT CALLUS 4. HARD CALLUS 5. BRIDGING 6. REMODELLING

6.2 KNH-UON ERC Approval Letter

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Ref: KNH-ERC/A/128

29th March, 2022

Dr. Martin Kimani Kaggia
Reg. No.H58/6868/2017
Dept. of Orthopaedic Surgery
Faculty of Health Sciences
University of Nairobi

KENYATTA NATIONAL HOSPITAL APPROVED 29 MAR 2022 KNH/UoN-ERC P.O. Box 20723 - 00202 NAIROBI

Dear Dr. Kaggia,

RESEARCH PROPOSAL: CORRELATION BETWEEN PEAK TRANSFORMING GROWTH FACTOR BETA 1 PLASMA LEVELS AND EARLY STAGE OF RADIOLOGICAL UNION IN PAEDIATRIC SUPRACONDYLAR HUMERUS FRACTURES AT KENYATTA NATIONAL HOSPITAL (P929/12/2021)

This is to inform you that KNH-UoN ERC has reviewed and approved your above research proposal. Your application approval number is **P929/12/2021.** The approval period is 29th March 2022 – 28th March 2023.

This approval is subject to compliance with the following requirements;

i. Only approved documents including (informed consents, study instruments, MTA) will be used.
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by KNH-UoN ERC.
iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to KNH-UoN ERC 72 hours of notification.
iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH-UoN ERC within 72 hours.
v. Clearance for export of biological specimens must be obtained from relevant institutions.
vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
vii. Submission of an executive summary report within 90 days upon completion of the study to KNH-UoN ERC.

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://research-portal.nacosti.go.ke and also obtain other clearances needed.

Yours sincerely,

DR. BEATRICE K.M. AMUGUNE
SECRETARY, KNH-UoN ERC

c.c. The Dean, Faculty of Health Sciences, UoN
The Senior Director, CS, KNH
The Chairperson, KNH- UoN ERC
The Assistant Director, Health Information, KNH
The Chair, Dept. of Orthopaedic Surgery, UoN
Supervisors: Prof. Atinga John Ernest Oluoch, Dept. of Orthopaedic Surgery, UoN
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