THE UTILITY OF BASIC FIBROBLAST GROWTH FACTOR AS A NON-INVASIVE BIOMARKER OF FOCAL SEGMENTAL GLOMERULOSCLEROSIS IN HIV POSITIVE AND NEGATIVE CHILDREN

By

Nokwanda Zamahlubi Gumede

Submitted in partial fulfillment for the degree of

MASTER OF MEDICAL SCIENCE

in the

Discipline of Clinical Medicine

Nelson R Mandela School of Medicine

College of Health Sciences

University of KwaZulu-Natal

Durban, South Africa

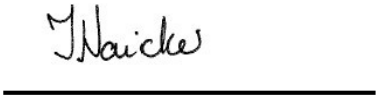
2018

PREFACE

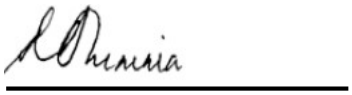
This study represents original work by the author and has not been submitted in any other form to another University. Where use was made of the work of others, it has been duly acknowledged in the text.

The research described in this dissertation was carried out in the Optics & Imaging Centre, Doris Duke Medical Research Institute, College of Health Sciences, University of KwaZulu-Natal, Durban, South Africa under the supervision of Professors Thajasvarie Naicker and Rajendra Bhimma.

Nokwanda Gumede



Thajasvarie Naicker (co-supervisor)



Rajendra Bhimma (supervisor)

DECLARATION

I, **Nokwanda Zamahlubi Cele** declare that:

(i) The research reported in this dissertation, except where otherwise indicated is my original work.

(ii) This dissertation has not been submitted for any degree or examination at any other university.

(iii) This dissertation does not contain other person's data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.

(iv) This dissertation does not contain other persons writing, unless specifically acknowledged as being sourced from other researchers. Where other sources have been quoted, then:

a) Their words have been rewritten but the general information attributed by them has been referenced.

b) Where their exact words have been used their writing had been placed inside quotation marks and referenced.

(v) Where I have reproduced a publication of which I am an author, co-author, I have indicated in detail which part of the publication was actually written by myself alone and have fully referenced such publications.

(vi) This dissertation does not contain text, graphics, or tables copied and pasted from the internet, unless specifically acknowledged and the source being detailed in the dissertation and the reference sections.

Signed: _____ Date: 03/08/03

DEDICATION

To my grandmother, Lindiwe Cele - You have always been my pillar of strength. You are the woman behind all my achievements and you the reason why giving up has never been an option. Thank you for your support and words of wisdom. I hope this dissertation will be an inspiration to my children as well as my grandchildren.

To my mother and father - Bongiwe and Mlaleni Yengwa; if it was not for you I will not be where I am today. Thank you for your understanding, patience and for making me realize my potential.

Family and Friends - My deepest gratitude and special thanks to my loved ones, for all their prayers and continued support.

To God - Above all, I thank the living God Almighty for granting me this opportunity and strength to successfully complete this research project. I could never have done this without the faith I have in you.

"Therefore I tell you, whatever you ask for in prayer, believe that you have received it, and it will be yours."

(Mark 11:24)

ACKNOWLEDGEMENTS

- Special gratitude to my supervisors Professors T Naicker and R Bhimma for their support, patience, valuable and constructive advice.

- To all my lab members, thank you for your technical assistance and for creating a warm working atmosphere.

- My deepest gratitude and special thanks to my loved ones, grandmother, my mother and my friends for all their prayers and continued support.

- Lastly, thank you to the National Research Foundation (South Africa) and College of Health Sciences for financial support.

- Above all, I thank the living God Almighty for granting me this opportunity and strength to successfully complete this research project.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

HIV - Human Immunodefiency Virus

HIVAN - HIV-associated Nephropathy

FSGS - Focal segmental glomerulosclerosis

bFGF - Basic fibroblast growth factor

HSPG - Heparin sulfate proteoglycans

LIST OF TABLES

Chapter 2 –Manuscript

LIST OF FIGURES

Chapter 1

Chapter 2

ABSTRACT

Background: The human immunodeficiency virus (HIV) infection can lead to the development of HIVAN with the majority of patients progressing to end-stage kidney disease. Importantly, individuals of African ancestry are more at risk of developing HIVAN than their European descent counterparts. Early diagnosis and immediate nephrology referral are key steps in management because this enable predialysis education, allows for implementation of preventive measures that delay or even halt progression of HIVAN to end-stage kidney disease, as well as decrease morbidity and mortality. Currently, the diagnosis of HIVAN requires a kidney biopsy. Due to the development of genomics, epigenetics, transcriptomics, proteomics, and metabolomics, the introduction of novel techniques will allow for the identification of novel biomarkers in kidney diseases. Previous studies have recognized basic fibroblast growth factor (bFGF) as a biomarker for HIVAN, since significant levels of bFGF low affinity receptors have been previously found in the kidneys from HIV infected children. bFGF is an angiogenic growth factor that is involved in kidney growth and the pathogenesis of kidney diseases.

The aim of this study was to investigate the use of basic fibroblast growth factor (bFGF) as a non-invasive biomarker for the diagnosis of primary FSGS and HIVAN

Method: The study group consisted of 31 children; HIVAN (n=11) and idiopathic FSGS (n=20). The control group consisted of 40 children with no kidney disease; HIV positive (n=20) and HIV negative (n=20). Serum samples were stored at -80^{0}C and were analysed for bFGF using the Bio-Plex Pro^{TM} Human Cytokine. Statistical analysis was performed using GraphPad Prism version 5.

Results: The concentration of bFGF was higher in the HIVAN (mean= 9.0 ng/ml; 95% CI: 10.18 – 7.18) vs idiopathic FSGS (mean= 7.0 ng/ml; 95% CI: 8.16 – 6.59) (Mann-Whitney U= 66.5; *p*= 0.0685). There was a significant elevation of serum bFGF Mean Flourescence Intensity (MFI) in children with HIVAN when compared to controls [HIV positive (mean = 7.0 ng/ml; 95% CI: 7.56 – 6.38) (Mann-Whitney U= 58.8 ; *p*= 0.004) and negative controls (mean= 6.5 ng/ml; 95% CI: 7.00-6.06) (Mann-Whitney U= 43.5 *p*= 0.029)]. There was no statistically significant differences in serum bFGF MFI between patients with HIVAN vs. idiopathic FSGS and between idiopathic FSGS vs. controls.

Conclusion: This study demonstrated statistically significant difference between bFGF levels in children with HIVAN and controls, although it failed to distinguish statistically significant differences in bFGF levels between HIVAN and idiopathic FSGS.

CHAPTER ONE

BACKGROUND AND LITERATURE REVIEW

1.1 Basic fibroblast growth factor

The fibroblast growth factor (FGF) family consists of 18 secreted ligands. These secreted ligands can be categorized into two subfamilies; the canonical FGFs (FGF1-10, 16-18 and 20), and the hormone-like FGFs (FGF 19, 21 and 23) (Burgess and Maciag, 1989). FGFs are classified as paracrine, intracrine and endocrine FGFs based on their mode of action (Itoh *et al.*, 2015) . Paracrine and endocrine FGFs are secreted signaling molecules acting through cell-surface FGF receptors (FGFRs) (Ornitz and Itoh, 2015). In comparison, several endocrine FGFs, require α-Klotho or β-Klotho as a cofactor for binding to FGFRs (Ornitz and Itoh, 2015).

The phenotypes of endocrine FGF knockout mice demonstrate that FGF are involved in phosphate and vitamin D metabolism (Itoh *et al.*, 2015). There is evidence to suggest that endocrine FGF have a pathophysiological role in genetic and metabolic diseases. Abnormal levels of endocrine FGF may serve as a potential risk factor for metabolic diseases but also are useful biomarkers for detection of metabolic diseases (Itoh *et al.*, 2015). These discoveries provide new perceptions into the physiological and pathophysiological roles of endocrine FGFs and their role as potential biomakers and therapeutic targets for metabolic diseases.

FGF2, also known as basic fibroblast growth factor (bFGF) and FGF-β, is a growth factor and signaling protein encoded by the FGF2 gene (Dionne et al., 1990). It is synthesized primarily as a 155 amino acid polypeptide, resulting in an 18 kDa protein (Kim, 1998). Basic FGF as well as their receptors control certain key behaviours such as migration, differentiation, survival and proliferation (Brooks *et al.*, 2012). Furthermore, bFGF

specifically promotes fibroblast proliferation and up-regulates the expression of proliferation-associated genes (Strutz *et al*., 2000).

1.1.1 Molecules that mediate the function of FGF

The activated FGFs transfer their signals to cellular targets via four FGF receptors (FGFRs) (Ornitz and Itoh, 2016). A number of alternative splicing occur in different intracellular and extracellular encoding regions (Ornitz and Itoh, 2015). Furthermore, alternative splicing within the third immunoglobulin-like domain of FGFRs 1-3 generating b and c alternates are mainly important ligand binding specificity determinants. Remarkably, FGF1 is the only ligand that is capable of activating all FGFRs regardless of alternative splicing. It has been demonstrated by ligand binding specificity assays that the FGF7 subfamily specifically binds and stimulates a splice variant (Ornitz *et al.,* 1996). Uniquely, the FGF9 subfamily also stimulates FGFR 3b. As for the canonical FGF subfamilies (FGF 1, 4, 7, 8, 9) heparan sulfate (HS) acts as the primary endogenous co-factor for the activation of the receptor as well as its ligand binding. Lastly, intracellular the FGFs subfamily specifically bind and mediate *Nar* channels without activating FGFRs (Ornitz and Itoh, 2016).

Often FGF signals reciprocally and directionally through epithelial mesenchymal boundaries (Olsen *et al*., 2003). The functionality of these signaling pathways need exceedingly tight mediation of FGF activity as well as receptor specificity. Directional signaling mediates the patterning and outgrowth of a given compartment during development. Hence, differential expression of the receptors' alternate splice forms can inhibit or limit autocrine signaling (Olsen *et al.*, 2003).

1.1.2 Interaction with heparin or heparan sulfate

One of the important features of FGF is its interaction with heparan sulfate proteoglycan (HSPG) (Ornitz and Itoh, 2016). These interactions are responsible for the stabilization of

FGFs to inhibit proteolysis and thermal denaturation; thereby strictly preventing their release and diffusion into interstitial spaces (Virag *et al*., 2007). Interaction between HS and FGFs lead to the formation of higher order oligomers and dimers (Nusayr *et al.*, 2013). A study by (Schreiber *et al.*, 1985) demonstrated that HS increases the half-life and affinity of the FGF-FGFR complex and that HS can bridge FGF 2 including the FGFR by attaching to a groove created by the heparan-binding sites of both the receptor and ligand.

1.1.3 FGF in functional and structural kidney damage

Recent studies have established the intrinsic capacity of the kidney to undergo repair subsequent to acute damage via re-expression or repair proteins (Villanueva *et al*., 2014). Initiation with bFGF could speed up this process. Nonetheless, there is not sufficient evidence that supports whether bFGF can induce this phenomenon in damaged kidney cells (Villanueva *et al*., 2014).

bFGF is a protein that is released for the induction of cellular aggregation (Karavanova *et al*., 1996). The core effect of bFGF includes maintaining WT-1 synthesis, a transcription factor that is responsible for inducing the conversion of mesenchymal cells into metanephric tissue (Karavanova *et al*., 1996). Furthermore, bFGF is able to induce expression of repair proteins and speed up the process of repairing tissue when it is exogenously added or conversely, renal injury could be sustained when it is inhibited (Villanueva *et al*., 2008). Moreover, it has been established that bFGF stimulates the proliferation rate of kidney cells (Dudley *et al*., 1995).

Previous studies have recognized bFGF as a biomarker for renal disease, particularly in focal segmental glomerulosclerosis (FSGS), where they demonstrated significant levels of bFGF low affinity receptors in the kidney of HIV infected children (Ray *et al*., 1999). These studies

highlighted that bFGF in circulation is subsequently harboured in the kidney bound to HSPG (Klagsbrun and Baird, 1991).

1.1.4 Progression of kidney disease

In both animal models and in humans with FSGS, glomerulosclerosis is categorized by scarring of small sections of individual glomeruli as well as tubular structure (D'Agati *et al.,* 2011). The glomeruli show loss of capillaries leading to localized areas of progressive scarring and cell proliferation that ultimately result in breaking down of the capillary bed (Klahr and Morrissey, 2000). Tubules, mainly those bound to scarred glomeruli, are shrunken and in most cases, are delimited by inflammatory cells (Mullins *et al*., 2016) . This is followed by activation and proliferation of matrix-producing fibroblasts, mainly bFGF which synthesize substantial amounts of extracellular matrix deposited in an organized manner (Klahr and Morrissey, 2000). Several studies have established that the differential expression of certain bFGFs is one of the key reasons for scar development (Klahr and Morrissey, 2000). Thus, bFGF may play an important role in the scarring process pathognomic to FSGS.

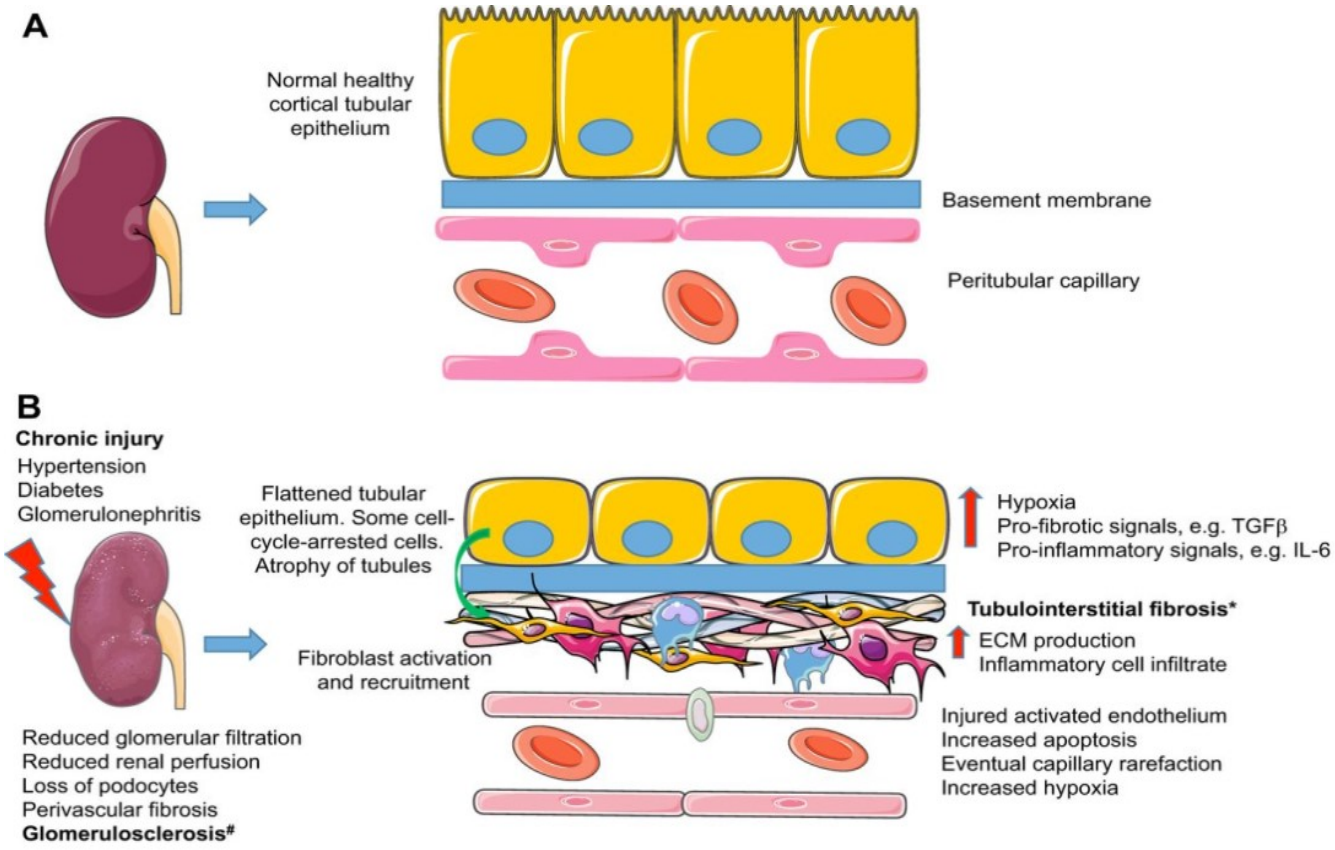


Figure 1: The pathophysiological processes linked to FSGS. (A) A healthy, normal kidney (left), and an enlarged view of the tubule its associated vasculature (right). (B) A chronically diseased kidney, displaying the progression of factors that lead to tubule-interstitial fibrosis (Mullins *et al*., 2016).

1.2 Focal segmental glomerulosclerosis

FSGS is one of the most common forms of steroid resistant nephrotic syndrome and is categorized histologically by obliteration of glomerular capillaries, mesangial sclerosis, foam cells, hyalinosis and adhesion between the Bowman's capsule and glomerular tuft (Rood *et al*., 2012). It is a worldwide public health issue due to its increasing incidence and prevalence. Nephrotic patients with FSGS have a poor prognosis with 50% progressing to end stage renal disease (ESRD) over 3 to 8 years (Korbet, 2000).

FSGS may be primary or secondary to various causes including infections such as Human Immunodeficiency virus type I (HIV-1) (Kim *et al*., 2016). Over the past 20 years there has been a significant increase in the incidence of FSGS, particularly in adult patients where there has been a 2-3 fold rise in the rate of diagnosis of FSGS (Kiffel *et al.*, 2011). A similar increase has been documented in children (Kiffel *et al*., 2011). Research has shown that majority of steroid resistant nephrotic syndrome is due to increasing levels of angiotensin II, which together with other growth factors, also up regulate the expression of basic fibroblast growth factors (bFGF) (Klahr and Morrissey, 2000). Hence, the development and progression of FSGS is determined by the action of these growth factors. FGFs are involved in the regulation of the balance between proliferation and cell growth versus apoptosis, necrosis and cell death as well as the balance of degradation against matrix accumulation (Fogo, 2015). Hence, glomerulosclerosis may result due to an increase in bFGF expression leading to the accumulation of extracellular matrix which contribute to renal malfunction (Fogo, 2015).

In patients with FSGS, bFGF is up regulated (Strutz *et al*., 2000). This is supported by a study done on HIV positive children where elevated levels of bFGF were observed and this was explained by the fact that renal HSPG functions as a sink, trapping bFGF as well as other growth factors (Strutz *et al*., 2000).

Globally, the incidence of FSGS has significantly increased over the past 30 years across all ethnic groups (Kiffel *et al.*, 2011). It is one of the most significant clinical disorders that causes acquired chronic kidney disease (CKD) in both adults and children (Kiffel *et al.*, 2011). FSGS has been the subject of intensive basic medico-clinical studies because of the significant morbidity arising from this condition. It was initially diagnosed on kidney biopsy specimens taken from patients suffering from steroid resistant nephrotic syndrome (Kiffel *et al*., 2011). There is not significance data on the epidemiology of FSGS in many parts of Africa due to the unavailability of renal registries. In Africa, glomerular diseases have been

reported to account for a large proportion of patients. Most renal biopsies reported from Africa originates from studies in North Africa (Okpechi *et al*., 2016). Of the 12,093 biopsies that were reported, 70% were from studies carried out in North Africa and these countries had more papers published after the year 2000 compared to countries from sub-Sahara Africa (Okpechi *et al*., 2016).

Attempts have been made to establish a histological classification of renal pathology so as to predict pharmacological response and treatment. In most of the pathological scoring systems used worldwide, the scarring associated with FSGS is characterised into five categories; collapsing, perihilar, not otherwise specified (NOS), hypercellular, and tip lesions (D'Agati, 2003). The intensity of mesangial cellularity can differ and there is continuing debate whether the severity of this observation has prognostic implications (Stokes *et al.*, 2006). The collapsing variant, which is subtype of FSGS, has constantly been proven to be resistant to treatment and runs a severe to moderately aggressive course (Kiffel *et al*., 2011).

Ultrastructural studies display occasional immune deposits, segmental scarring, and fusion of the podocyte foot process (Markowitz *et al*., 2003). Reticular inclusion bodies are observed on various secondary types of FSGS such as HIV nephropathy. Foot process effacement is often more severe and prevalent in primary FSGS than in secondary FSGS (Kiffel *et al.*, 2011). Immunofluorescence findings often display deposition of IgM and complement factor C_3 in sclerotic portions but this is reflected as a non-specific finding (Jennette and Hipp, 1985).

1.2.1 Pathogenesis of focal segmental glomerulosclerosis

Podocyte dysfunction is currently one of the central features in the pathogenesis of FSGS (Barisoni *et al*., 2009). Podocyte dysfunction could be due to an inherent abnormality within the podocyte or an exogenous element that stimulates podocyte damage. Loss of these non-

proliferative terminally differentiated cells is also critical in the progression of FSGS (Kiffel *et al*., 2011). *In vivo* experimental models that categorise podocyte damage and loss, showed that podocytopenia is largely related with the histological pattern of damage (Wharram *et al*., 2005). In experiment models that were performed it was observed that, if ≤20% podocytes are lost, resident glomerular epithelial stem cells transit from a position adjacent to the Bowman's space into the glomerulus to recover injured podocytes that were damaged by apoptosis and necrosis. However, if the loss is between 20-40%, FSGS lesions result, whereas if loss of podocyte is >40%, then there is global sclerosis of the glomerulus (Kiffel *et al.*, 2011).

Transformed expression of various signalling molecules are linked with podocyte damage and the progression of glomerulosclerosis in animal models of FSGS (Kaufman *et al*., 2010). These include Sidekick-1 and Notch proteins which play integral roles in the morphology of the glomerulus and normal nephrogenesis (Kaufman *et al*., 2010). Upcoming genomic studies may assist in determining the role of these as well as other molecules in the broad spectrum of disease severity that occur in FSGS patients.

One of the interesting improvements that have been discovered in the past decade in understanding FSGS is the association between genetic mutations and several structural proteins of the podocyte (Kaufman *et al.*, 2010). The first FSGS genetic studies revealed the occurrence of glomerulopathy in humans with altered genes for podocin included *NPHS1, NPHS2, WT-1, LAMB2, CD2AP, TRPC6, ACTN4* and *INF2* (Chen and Liapis, 2015). Furthermore, seven other genes have been demonstrated in FSGS family pedigrees which include; I*FN2, CD2AP, α-actinin -4, laminin β2, Wilms tumor-1, phospholipase C epsilon* (Kiffel *et al*., 2011). In addition, nephron protein mutations, which were initially demonstrated in connection with congenital nephrotic syndrome, can also result in steroid-resistant nephrotic syndrome in adults and children (Philippe *et al*., 2008). Generally, there

are two forms of mutations, autosomal dominant disease with onset in adulthood or in late adolescence and an autosomal recessive pattern of inheritance seen mainly in children (Philippe *et al.*, 2008). The incidence of sporadic FSGS cases related with each mutation differs from 3-20% depending on the patient cohort being tested. Current studies with genotype-phenotype correlations have simplified certain mutations that have an effect on the clinical course of disease in FSGS patients (Caridi *et al*., 2009). For instance, the *NPHS2* gene in podocin are related with onset of disease earlier in childhood and rapid progression to end stage kidney disease (Caridi *et al*., 2005).

1.2.2 Therapy

There is dearth of clinical information describing the outcome of randomized trials (RCT) in glomerular and nephrological diseases to guide treatment of FSGS (Leaf *et al.,* 2010). All FSGS patients profit from the use of antiproteinuric agents that antagonize the renin-angiotensin II-aldosterone axis(Kiffel *et al.*, 2011). This therapy comprises inhibition of renin by specific renin antagonists, aldosterone antagonists, angiotensin converting enzyme inhibitors, and lastly angiotensin receptor blockers(Mercier *et al*., 2014). There is emerging recognition of the role of T-cells in the pathogenesis of the disease, and the successive focus on immunosuppressive agents as possible treatments may be misguided and delay the search for therapeutic agents targeting T-cells (Meyrier, 2009). This is supported by current data that cyclosporine may be responsible for reducing proteinuria in FSGS animal models by acting specifically on the podocyte and maintaining the actin cytoskeleton instead of aiming at immunological mechanisms, specifically inhibition of nuclear factor of triggered T-cell (NFA7) (Meyrier, 2009).

About 25% of FSGS patients have total or partial remission of proteinuria after a course of corticosteroids (Meyrier, 2009). One exception to this typical grim outcome is the feedback

from a single center signifying that almost 80% (18/23) of children suffering from FSGS may accomplish a thorough or partial remission over a mean continuation period of 46 ± 5 months in response to extended treatment with pulses doses of methylprednisolone (Mendoza *et al.*, 1990). Using oral dexamethasone pulses, Kopp *et al* (unpublished data) tried to duplicate this in adults; however the outcomes were not as encouraging as reported in children by (Kiffel *et al.*, 2011). Hence, steroids are not consistently optimal therapy in FSGS patients. Furthermore, the major side effects of prolonged use of steroids significantly restricts its use in patients suffering from bone disease, hypertension, or those that are overweight (Meyrier, 2009).

The effectiveness of rituximab to encourage a remission in 25-40% of patients with steroid-resistant FSGS suggests that B-cells rather than T-cells may be possible immunosuppressive therapy targets (Kiffel *et al*., 2011). Additionally, other therapies that could be utilized include, mycophenolate mofetil (MMF) which is one of the most commonly used immunosuppressive drugs used either alone or in combination with other immunosuppressive drugs (e.g. corticosteroids and/or calcineurin inhibitors) for the prevention of organ rejection after solid organ transplantation as well as in the therapy of autoimmune and neoplastic diseases (Oellerich *et al.*, 2000, Allison and Eugui, 2000) .

1.2.3 Adjunctive therapies for FSGS

Several adjunctive therapies have been proposed in an attempt to reduce the progression of tissue injury in FSGS. These include angiotensin I-converting enzyme inhibitors, renin-angiotensin-aldosterone, angiotensin receptor blockers (ARBs) and statins. However, these therapies have not displayed significant positive improvements specifically in tubular function and glomerular filtration rates that could prevent the final phase of renal disease

(Wolf *et al.*, 2003). Hence, research for new promising and alternative treatments is one of the main concerns in nephrology.

Statins are well known to decrease cardiovascular morbidity and mortality in patients with and without recognized cardiovascular disease as well as in several high-risk populations (Shepherd *et al.*, 1995). Nonetheless, definite evidence for developed cardiovascular outcomes with statin therapy for renal disease is not yet available (Agarwal, 2007).

The overall conclusion established from various FSGS researchers suggests that suitable pharmacological treatments for the development of renal fibrosis may require an approach that is multi-pharmacological (Kiffel *et al.*, 2011). More research is required to understand the developmental role of bFGF and other FGFs as these will provide further insight as to whether a single or multiple FGFs interact during the progression of FSGS. The aim of this study is to investigate bFGF as an non-invasive biomaker to renal biopsy in children with primary FSGS and HIVAN compared to controls.

1.2.4 Aims and Objectives

Aim

The aim of this study was to investigate the use of basic fibroblast growth factor (bFGF) as a non-invasive biomarker for the diagnosis of primary FSGS and HIVAN.

Objectives

1. To compare the levels of bFGF in children with biopsy proven FSGS to healthy controls.
2. To measure and compare the levels of bFGF in FSGS according to HIV status.
3. To compare the levels of bFGF in FSGS and HIVAN to HIV positive children with no kidney disease and healthy controls.

CHAPTER TWO

South African Journal of Child Health

THE ROLE OF BASIC FIBROBLAST GROWTH FACTORS AS A BIOMARKER OF FOCAL SEGMENTAL GLOMERULOSCLEROSIS IN HIV POSITIVE AND NEGATIVE CHILDREN

--Manuscript Draft--

Manuscript Number:	SAJCH1589
Article Type:	Original Research
Keywords:	HIV; HIVAN; bFGF; FSGS
Corresponding Author:	Nokwanda Zamahlubi Gumede, Mmed University of KwaZulu-Natal College of Health Sciences SOUTH AFRICA
First Author:	Nokwanda Zamahlubi Gumede, Mmed Sci
Order of Authors:	Nokwanda Zamahlubi Gumede, Mmed Sci
	Rajendra Bhimma, MBChB , Masters in Medicine
	Thajasvarie Naicker, BSc. , MMed Scie, PhD
Manuscript Region of Origin:	SOUTH AFRICA
Abstract:	Abstract Background: The human immunodeficiency virus (HIV) infection can lead to the development of HIVAN with the majority of patients progressing to end-stage kidney disease. Previous studies have recognized basic fibroblast growth factor (bFGF) as a biomarker for HIVAN, since significant levels of bFGF low affinity receptors have been previously found in the kidneys from HIV infected children. The objective of the study was to determine the role of bFGF in the development of focal segmental glomerulosclerosis (FSGS) in HIV positive and negative children. Method: The study group consisted of 31 children; HIVAN (n=11) and idiopathic FSGS (n=20). The control group consisted of 40 children with no kidney disease; HIV positive (n=20) and HIV negative (n=20). Serum samples were stored at -800C and were analysed for bFGF using the Bio-Plex ProTM Human Cytokine. Results: The concentration of bFGF was higher in the HIVAN (mean= 8.682ng/ml; 95% CI: 10.18 - 7.185) vs idiopathic FSGS (mean= 7.375ng/ml; 95% CI: 8.16 - 6.59) (Mann-Whitney U= 66.5; p= 0.0167). There was a significant elevation of serum bFGF Mean Flourescence Intensity (MFI) in children with HIVAN when compared to controls [HIV positive (mean = 6.975ng/ml; 95% CI: 7.565 - 6.385) (Mann-Whitney U= 58.8 ; p= 0.0288) and negative controls (mean= 6.538ng/ml; 95% CI: 7.006-6.069) (Mann-Whitney U= 43.5 p= 0.0043)]. Conclusion: This study demonstrated statistically significant difference between bFGF levels in children with HIVAN and controls, although it failed to distinguish statistically significant differences in bFGF levels between HIVAN and idiopathic FSGS. Keywords: HIV, HIVAN, bFGF, FSGS Running title: FGF levels in HIV associated nephropathy

The utility of basic fibroblast growth factor as a non-invasive biomarker of focal segmental glomerulosclerosis in HIV positive and negative children

Authors: [a]Nokwanda Gumede, [b]Thajasvarie Naicker and [a]Rajendra Bhimma

Affiliations: [a]Clinical Medicine, College of Health Sciences, University of KwaZulu-Natal, Durban, KwaZulu-Natal, South Africa, Department of Pediatrics & Child Health, Nelson R. Mandela School of Medicine, University of KwaZulu-Natal, Durban, KwaZulu-Natal, South Africa

***Corresponding author:**
Nokwanda Gumede
Pediatrics & Child Health,
College of Health Sciences,
University of KwaZulu-Natal,
Durban, South Africa.
Postal address: Private Bag 7
Congella
KwaZulu-Natal
4013
South Africa
Email address: nokwahcele@gmail.com;bhimma@ukzn.ac.za; naickera@ukzn.ac.za

Total word count:
Word count excluding abstract and references:
Number of figures: 7
Number of tables: 1

Abstract

Background: The human immunodeficiency virus (HIV) infection can lead to the development of HIVAN with the majority of patients progressing to end-stage kidney disease. Importantly, individuals of African ancestry are more at risk of developing HIVAN than their European descent counterparts. Early diagnosis and immediate nephrology referral are key steps in management because this enable predialysis education, allows for implementation of preventive measures that delay or even halt progression of HIVAN to end-stage kidney disease, as well as decrease morbidity and mortality. Currently, the diagnosis of HIVAN requires a kidney biopsy. Due to the development of genomics, epigenetics, transcriptomics, proteomics, and metabolomics, the introduction of novel techniques will allow for the identification of novel biomarkers in kidney diseases. Previous studies have recognized basic fibroblast growth factor (bFGF) as a biomarker for HIVAN, since significant levels of bFGF low affinity receptors have been previously found in the kidneys from HIV infected children. bFGF is an angiogenic growth factor that is involved in kidney growth and the pathogenesis of kidney diseases.

The aim of this study was to investigate the use of basic fibroblast growth factors (bFGF) as a non-invasive biomarker for the diagnosis of FSGS in HIV positive (HIVAN) and HIV negative children (primary FSGS).

Method: The study group consisted of 31 children; HIVAN (n=11) and idiopathic FSGS (n=20). The control group consisted of 40 children with no kidney disease; HIV positive (n=20) and HIV negative (n=20). Serum samples were stored at -80^{0}C and were analysed for bFGF using the Bio-Plex Pro^{TM} Human Cytokine. Statistical analysis was performed using GraphPad Prism version 5.

Results: The concentration of bFGF was higher in the HIVAN (mean= 9.0 ng/ml; 95% CI: 10.18 – 7.18) vs idiopathic FSGS (mean= 7.0 ng/ml; 95% CI: 8.16 – 6.59) (Mann-Whitney U= 66.5; *p*= 0.0685). There was a significant elevation of serum bFGF Mean Flourescence Intensity (MFI) in children with HIVAN when compared to controls [HIV positive (mean = 7.0 ng/ml; 95% CI: 7.56 – 6.38) (Mann-Whitney U= 58.8 ; *p*= 0.004) and negative controls (mean= 6.5 ng/ml; 95% CI: 7.00-6.06) (Mann-Whitney U= 43.5 *p*= 0.029)]. There was no statistically significant differences in serum bFGF MFI between patients with HIVAN vs. idiopathic FSGS and between idiopathic FSGS vs. controls.

Conclusion: This study demonstrated statistically significant difference between bFGF levels in children with HIVAN and controls, although it failed to distinguish statistically significant differences in bFGF levels between HIVAN and idiopathic FSGS.

Keywords: HIV, HIVAN, bFGF, FSGS

Running title: FGF levels in HIV associated nephropathy

Introduction

Human immunodeficiency virus (HIV) infected antiretroviral therapy naïve children display a high viral load during the late phase of infection, placing them at risk of developing several types of kidney diseases, including HIV associated nephropathy (HIVAN) [1]. However children on antiretroviral therapy, kidney disease is usually uncommon [2]. The spectrum of kidney disease that occurs with HIV infection includes acute kidney injury, disorders of tubular function, thrombotic microangiopathies, kidney injury secondary to drug use and various forms of chronic glomerular diseases including HIVAN and HIV associated immune complex disorders [3] . The classical findings of HIVAN include: persistent proteinuria usually accompanied by varying degrees of haematuria, urinary sediment with urinary microcysts, azotemia, normal to large kidneys on ultrasound images, normal blood pressure, and focal segmental glomerulosclerosis (FSGS) on kidney biopsy findings [4,2]. During HIV infection, FSGS is an important comorbidity [5].

FSGS is a primary cause of nephrotic syndrome in children, and if untreated, has a poor prognosis [6]. Nonetheless, the pattern of segmental and focal sclerosis is not specific to disease with the primary podocyte lesions, and several other diseases e.g. HIVAN, demonstrate light microscopic lesion patterns such as overlying cell hyperplasia as well as glomerular tuft injuries similar to primary FSGS [7].

In the pre-antiretroviral therapy era, HIVAN was characterized by rapid progression to end-stage kidney disease leading to the need for dialysis [8]. Highly active antiretroviral therapy (HAART) has improved the natural course of this disease, increasing the importance of early diagnosis and allied suitable care [8]. Robust biomarkers may assist in diagnosing and monitoring kidney disease commencing from initial stages of the disease [9]. Previous studies have recognized bFGF as a biomarker for FSGS, since significant levels of bFGF low affinity receptors have been previously found in the kidney of HIV infected children [10]. bFGF is an

angiogenic vascular growth factor, that is essential for cellular aggregation induction [11]. It is involved in the stimulation of epithelial condensation, apoptosis inhibition as well as maintenance of WT-1 synthesis [12]. Furthermore, bFGF is mainly stored as an inactive pool within the extracellular matrix and vessel wall, and is absent in the circulation unless it is secreted during tissue injury and angiogenesis [13]. Elevated levels of bFGF may result in the progression of FSGS, and unrestrained elevation of bFGF in the kidney extracellular matrix can generate the fibrotic lesions and tubulointerstial proliferative distinctive to HIVAN [13]. However, the processes that regulate secretion and behaviour of bFGF in glomeruli and renal tubules are as yet not clearly understood [14].

Based on the findings that elevated bFGF may result in the progression of FSGS and plays a role in the generation of fibrotic lesions and tubulintersitial proliferation distinct to HIVAN, our aim was to determine if bFGF can be used as a non invasive biomarker for the detection of HIVAN and idiopathic FSGS.

Method and Materials

Ethical consideration

This study was performed on blood samples collected from Black African children with idiopathic FSGS and HIVAN aged between 1-16 years. Ethical approval was received from the University of KwaZulu-Natal Biomedical Research Ethics Committee (BREC 220/17) and permission to perform the project was given by the Hospital managers at Inkosi Albert Luthuli Central Hospital and King Edward VIII Hospital. Furthermore, written informed consent was obtained from the children's parents or legal guardian prior to collection of blood samples. All identifies were removed and following collection, each blood sample was assigned a study identity number to maintain anonymity. All data collected from the hospital records on a Microsoft Excel spreadsheet using Windows (version 7)® was protected by a password only known by the researchers.

Diagnosis of HIVAN

The diagnosis of HIVAN was made following confirmation of HIV-1 infection and presence of persistent proteinura ≥1+ on urinary dipstick examination (on at least 3 separate occassions in non-febrile children) with one or more of the following: (i) presence of enlarged echogenic kidneys by renal ultrasound; (ii) abnormal urinary sediment; (iii) microcystic tubular dilation, a childhood variant of HIVAN in the absence of significant podocyte lesions; and (iv) histological finding of FSGS [23].

Creatinine measurement

Serum samples were analysed for creatinine using the modified Jaffe kinetic method on the Siemens Advia 1800 analyser (Siemens Healthcare Diagnostics, Tarrytown, USA), The revised Schwartz equation was used to estimate GFR using the equation [24].

Revised Schwartz GFR

$$= \frac{36.5 \times \text{height in cm}}{\text{creatinine in (umol/l)}}$$

The study population

Black South African children aged between 1-16 years from KwaZulu-Natal were recruited. The study population included children with biopsy proven HIVAN (n = 11) and idiopathic FSGS (n =20). A control group (n= 40) was further stratified based on the HIV status *i.e.*, HIV positive children with no kidney disease (n = 20) and HIV negative children with no kidney disease (n = 20).

Inclusion criteria for HIVAN group

Black South African children with HIV-associated biopsy-proven nephropathy (FSGS) aged between 1-16 years old who gave written in formed consent for participation in the study.

Exclusion criteria for HIV Associated FSGS (HIV positive) group

HIV negative Black South African with histological forms of HIV related nephropathy other than FSGS, children <1 year old, failure to obtain written informed consent for participation in the study, absence of kidney biopsy or inadequate tissue for histology.

Inclusion criteria for idiopathic FSGS (HIV Negative) group

Black South African children with biopsy-proven idiopathic FSGS aged between 1-16 years old with written informed consent for participation in the study.

Exclusion criteria for idiopathic FSGS (HIV Negative) group

HIV negative Black South African children between 1-16 years old with idiopathic nephrotic syndrome having histological forms of nephropathy other than FSGG, children <1 year old, failure to obtain written informed consent for participation in the study, or inadequate tissue for histology.

Inclusion criteria for control group

HIV negative and positive Black South African children between the ages of 1-16 years with no kidney disease.

Exclusion criteria for control group

Black South African children with FSGS or other kidney diseases and children below the age of 1 year or over 16 years.

Bioplex Immunoassay

Serum from stored samples were analysed for bFGF using the quantitative BioPlex Pro™ Human Cytokine. Briefly, samples as well as the standards were diluted using the Bioplex sample diluent HB and standard diluent HB, respectively. Coupled beads were added into each well of the 96 well assay plate, and a sealing tape was used to protect the beads from light. Using 100 µl of assay buffer, the plate was washed and left to incubate at 850 rpm at room temperature (RT). With 10 min left in the incubation, the detection antibodies were vortexed and added to each well, followed by a second incubation at 850 rmp at RT. To complete the reaction an addition of fluorescent conjugate streptavidin-phycoerythrin (SAPE) was added to the wells prior to incubation of the plate at RT. After the final wash, beads were resuspended in 125 µl assay buffer.

Analysis

Samples were analysed on Bio-plex MAGPIX^{TM} Multiplex system (Bio-Rad laboratories Inc., USA) and the data was obtained using the Bio-Plex Manager^{TM} version 4.1 software. A standard curve was generated using the known concentration (ng/ml) of each analyte by plotting the median fluorescent intensity (MFI) signal against concentration[39]. These standards were used to interpolate the concentration of the unknown samples. Intra-plate variability were determined with CV <20% and ($\frac{Observed\ concentration}{Expected\ concentration} X100$) between 70-130% (r=0.8, p=0.05). The data was imported into an Excel spreadsheet for statistical analysis.

Statistical analysis

Data was entered into SPSS version 24 (Statistical Packages for the Social Sciences) and GraphPad Prism version 5 (GraphPad software version 5, San Diego, Califonia, USA) for analysis. A *p* value <0.05 was considered as statistically significant. A descriptive statistical analysis of the data (means, standard deviations, ranges, frequencies and percentages, etc.) were initially conducted prior to inferential analysis. The independent samples T-Test test and Anova were used to determine if high levels of bFGFs are associated with the development of FSGS. The Chi-square test of association was used to assess any associations between categorical variables.

Results

Patient demographics and Clinical Characteristics

The overall study population was inclusive of 71 children, 31 of whom had a histopathological pattern of FSGS. A summary of the patients' demographics are outlined in

Table 1. The mean age for idiopathic FSGS and HIVAN was (9 ± 3.11 years) and (10 ± 3.62 years) respectively.

To evaluate associations with the variability, bFGF concentration was compared with urea, cholesterol, eGFR, CD4, age, weight and creatinine. Non-significant statistical correlations were demonstrated, therefore signifying that these demographic data had no observable effect on the bFGF MFI in children.

The Fluorescence Intensity (FI) of bFGF

The serum FI of bFGF is displayed in figures 1 – 7.

There was a significant elevation of serum bFGF MFI in children with HIVAN when compared to the HIV positive (mean = 7.0 ng/ml; 95% CI: 7.56 – 6.38) (Mann-Whitney U= 58.8 ; p= 0.004) and HIV negative (mean= 6.5 ng/ml; 95% CI: 7.00-6.06) (Mann-Whitney U= 43.5 p= 0.029) control groups. There was no statistically significant difference between children with idiopathic FSGS vs positive and negative controls.

Discussion

Several studies have confirmed the presence of HIVAN in children who are most likely to develop nephrotic syndrome in association with FSGS [10]. This study reports a significant elevation of serum bFGF MFI in children with HIVAN when compared to the HIV positive and HIV negative control groups (p = 0.0043). Similarly a study by *Liu et al*. detected elevated levels of bFGF in HIVAN children [13]. HIVAN is associated with an increased expression of renal HSPGs that may facilitate the accumulation of bFGF in the kidney and the progression to end-stage kidney disease. Additionally, renal tubular epithelial cells harvested from the serum of children with HIVAN produce and release high levels of bFGF, as well as a FGF-binding protein, that facilitates the release of several members of the FGF's family, including bFGF [13]. These findings may explain the elevated levels of serum bFGF in children with HIVAN. However, our study demonstrated no significant difference in paediatric bFGF between HIVAN versus idiopathic FSGS.

Furthermore, we noted higher albumin levels in HIVAN children compared to primary FSGS children. These results corroborate findings that suggest increased levels of albumin result from improved appetite and general well-being as well as decreased loss of protein in the urine. Proteinuria is a marker of HIV nephropathy and has been correlated with decreased renal function and progression to ESRD [14]. It must be noted that in our study all HIV infected children were on HAART treatment. HAART therapy and angiotensin-converting enzyme antagonists are highly effective in reducing proteinuria and protecting renal function [15].

In our study high levels of cholesterol and creatinine together with reduced eGFR were found in children with idiopathic FSGS compared to children with HIVAN. These parameters further support the view that children with HIV-associated FSGS treated with HAART may achieve full remission and delay or prevent progression to end-stage kidney disease. Such

findings are supported by a study on 152 biopsy proven HIVAN patients treated with HAART that were found to have better renal survival compared with patients who did not receive HAART. Hence, HIVAN should be considered as an indication to initiate HAART [16].

In our study, the bioactive protein, bFGF was significantly lower in HIV negative and positive controls compared to HIVAN children. The possible reason for the significantly raised bFGF in children with HIVAN compared to HIV positive and negative controls includes stimulation of cell growth by bFGF [17] [18]. High levels of bFGF may lead to the development of FSGS [13]. bFGF can increase the attachment of HIV-infected mononuclear cells to renal epithelial cells [19], enhance the expression of hypoxia-inducible genes in these cells [14], induce renal microcysts [20], and/or cause FSGS [21]. Excessive accumulation of bFGF in the renal extracellular matrix can induce tubulointerstitial proliferative and fibrotic lesions typical of HIVAN [13]. However, the mechanisms that control the release and activity of bFGF in renal tubules and glomeruli are not clearly described.

This study was based on previous studies displaying an advanced accumulation of heparin-binding growth factors in association with the occurrence of the renal microcytic tubular lesions that are biomarkers for children with HIVAN [18]. Another study that was done based on this approach that revealed significant differences in the levels of bFGF between HIVAN and the control group, with bFGF showing elevation. In support of the latter finding, our study also reports similar results [22].

In our study, no significant difference was observed between idiopathic FSGS vs. HIV positive and negative controls as well as between children having idiopathic FSGS vs. HIVAN. This lack of significant differences in bFGF levels between HIVAN study group and controls may be also be attributed to the small sample size that was used in our study or that

our patients had established disease and were on treatment, with arrested or markedly attenuated distal tubular cell injury.

Our study and that of Wyatt [19], demonstrate that bFGF can be used as a potential candidate renal biomarker for HIVAN. However, further studies in a larger cohort of children are necessary to validate these data. It is also essential to examine other new biomarkers for HIVAN as an approach to making a more conclusive diagnosis of HIVAN [24].

There are several other limitations to our study. Samples were collected and stored at -80^0C to prevent protein degradation until further use. It is likely that during the storage periods, there was a decrease of the serum proteins due to protein degradation. This could have led to the non-significant differences in serum levels of bFGF. Moreover, this study only included a homogenous group of Black African children and may therefore not be applicable to other population groups. Children recruited for the study were on treatment and this could have had an impact on the levels of serum levels of bFGF studied.

From the observations of the study the serum profile of bFGF was the highest in HIVAN, followed by idiopathic FSGS, and HIV positive controls, with lowest level found in HIV negative controls. The significant difference between bFGS in children with HIVAN compared to controls suggests that bFGF may be a useful biomarker in distinguishing HIVAN from HIV positive children with no kidney disease. In HIV-infected children, one can minimize the risk of developing kidney disease by beginning antiretroviral therapy early in the course of the disease and continue with the treatment. bFGF could be used as an adjunctive marker for the detection of kidney disease in HIV infected children although this study did not address the sensitivity or specificity of bFGF against proteinuria or microalbuminuria or other biomarkers for the early detection of HIVAN.

Conclusion

This study demonstrated statistically significant difference between bFGF levels in children with HIVAN and controls, although it failed to distinguish statistically significant differences in bFGF levels between HIVAN and idiopathic FSGS. Further studies with a larger samples size are required to assess the role of bFGF as a potential biomarker for the detection of HIVAN in children.

Acknowledgement

We appreciatively acknowledge the patients that consented to participate in this study, to King Edward VIII Hospitals and Inkosi Albert Luthuli nurses for their assistance during the sample collection. We would also like to thank the Optics and Imaging Centre at DDMRI of Nelson R Mandela School of Medicine in South Africa, for the use of the Bioplex equipment.

Funding

The authors would like to thank the College of Health Science UKZN and National Research Foundation, for financial support.

References

1. SOLER-GARCÍA, Á. A., RAKHMANINA, N. Y., MATTISON, P. C. & RAY, P. E. 2009. A urinary biomarker profile for children with HIV-associated renal diseases. *Kidney international,* 76**,** 207-214.

2. KALAYJIAN, R. C. 2010. The treatment of HIV-associated nephropathy. *Advances in chronic kidney disease,* 17**,** 59-71.

3. BHIMMA, R., PURSWANI, M. U. & KALA, U. 2013. Kidney disease in children and adolescents with perinatal HIV-1 infection. *Journal of the International AIDS Society,* 16.

4. WYATT, C. M. & KLOTMAN, P. E. 2009. HIV-associated nephropathy. *Genetic Diseases of the Kidney.* Elsevier.

5. HUSAIN, N. E., AHMED, M. H., ALMOBARAK, A. O., NOOR, S. K., ELMADHOUN, W. M., AWADALLA, H., WOODWARD, C. L. & MITAL, D. 2018. HIV-Associated Nephropathy in Africa: Pathology, Clinical Presentation and Strategy for Prevention. *Journal of clinical medicine research,* 10**,** 1.

6. ROY, S. & STAPLETON, F. B. 1987. Focal segmental glomerulosclerosis in children: comparison of nonedematous and edematous patients. *Pediatric Nephrology,* 1**,** 281-285.

7. KIFFEL, J., RAHIMZADA, Y. & TRACHTMAN, H. 2011. Focal segmental glomerulosclerosis and chronic kidney disease in pediatric patients. *Advances in chronic kidney disease,* 18**,** 332-338.

8. RÖLING, J., SCHMID, H., FISCHEREDER, M., DRAENERT, R. & GOEBEL, F. 2006. HIV-Associated Renal Diseases and Highly Active Antiretroviral Therapy—Induced Nephropathy. *Clinical Infectious Diseases,* 42**,** 1488-1495.

9. SONI, S. S., RONCO, C., KATZ, N. & CRUZ, D. N. 2009. Early diagnosis of acute kidney injury: the promise of novel biomarkers. *Blood purification,* 28**,** 165-174.

10. RAY, P. E., LIU, X.-H., XU, L. & RAKUSAN, T. 1999. Basic fibroblast growth factor in HIV-associated hemolytic uremic syndrome. *Pediatric Nephrology,* 13**,** 586-593.

11. KOPP, J. B. 2000. BMP receptors in kidney. *Kidney international,* 58**,** 2237-2238.

12. KOPP, J. B., SMITH, M. W., NELSON, G. W., JOHNSON, R. C., FREEDMAN, B. I., BOWDEN, D. W., OLEKSYK, T., MCKENZIE, L. M., KAJIYAMA, H. & AHUJA, T. S. 2008. MYH9 is a major-effect risk gene for focal segmental glomerulosclerosis. *Nature genetics,* 40**,** 1175.

13. LIU, X.-H., AIGNER, A., WELLSTEIN, A. & RAY, P. E. 2001. Up-regulation of a fibroblast growth factor binding protein in children with renal diseases. *Kidney international,* 59**,** 1717-1728.

14. VILLANUEVA, S., CONTRERAS, F., TAPIA, A., CARREÑO, J. E., VERGARA, C., EWERTZ, E., CESPEDES, C., IRARRAZABAL, C., SANDOVAL, M. & VELARDE, V. 2013. Basic fibroblast growth factor reduces functional and structural damage in chronic kidney disease. *American Journal of Physiology-Renal Physiology,* 306**,** F430-F441.

15. FREDRICK, F., FRANCIS, J. M., RUGGAJO, P. J. & MARO, E. E. 2016. Renal abnormalities among HIV infected children at Muhimbili National Hospital (MNH)—Dar es Salaam, Tanzania. BMC nephrology, 17, 30.

16. LU, T.-C. & ROSS, M. 2005. HIV-associated nephropathy: a brief review. *The Mount Sinai journal of medicine, New York,* 72**,** 193-199

17. OLAWUMI, H. & OLATUNJI, P. 2006. The value of serum albumin in pretreatment assessment and monitoring of therapy in HIV/AIDS patients. *HIV medicine,* 7**,** 351-355.

18. ATTA, M. G., GALLANT, J. E., RAHMAN, M. H., NAGAJOTHI, N., RACUSEN, L. C., SCHEEL, P. J. & FINE, D. M. 2006. Antiretroviral therapy in the treatment of HIV-associated nephropathy. *Nephrology Dialysis Transplantation,* 21**,** 2809-2813.

19. WYATT, C. M. 2017. Kidney disease and HIV infection. *Topics in antiviral medicine,* 25**,** 13.

20. KLAHR, S. & MORRISSEY, J. J. 2000. The role of vasoactive compounds, growth factors and cytokines in the progression of renal disease. *Kidney International,* 57**,** S7-S14.

21. TANG, P., JEREBTSOVA, M., PRZYGODZKI, R. & RAY, P. E. 2005. Fibroblast growth factor-2 increases the renal recruitment and attachment of HIV-infected mononuclear cells to renal tubular epithelial cells. *Pediatric Nephrology,* 20**,** 1708-1716.

22. LI, Z., JEREBTSOVA, M., LIU, X.-H., TANG, P. & RAY, P. E. 2006. Novel cystogenic role of basic fibroblast growth factor in developing rodent kidneys. *American Journal of Physiology-Renal Physiology,* 291**,** F289-F296.

23. RAMSURAN, D., BHIMMA, R., RAMDIAL, P. K., NAICKER, E., ADHIKARI, M., DEONARAIN, J., SING, Y. & NAICKER, T. 2012. The spectrum of HIV-related nephropathy in children. *Pediatric nephrology,* 27**,** 821-827.

24. SCHWARTZ, G. J., MUNOZ, A., SCHNEIDER, M. F., MAK, R. H., KASKEL, F., WARADY, B. A. & FURTH, S. L. 2009. New equations to estimate GFR in children with CKD. *Journal of the American Society of Nephrology,* 20**,** 629-637.

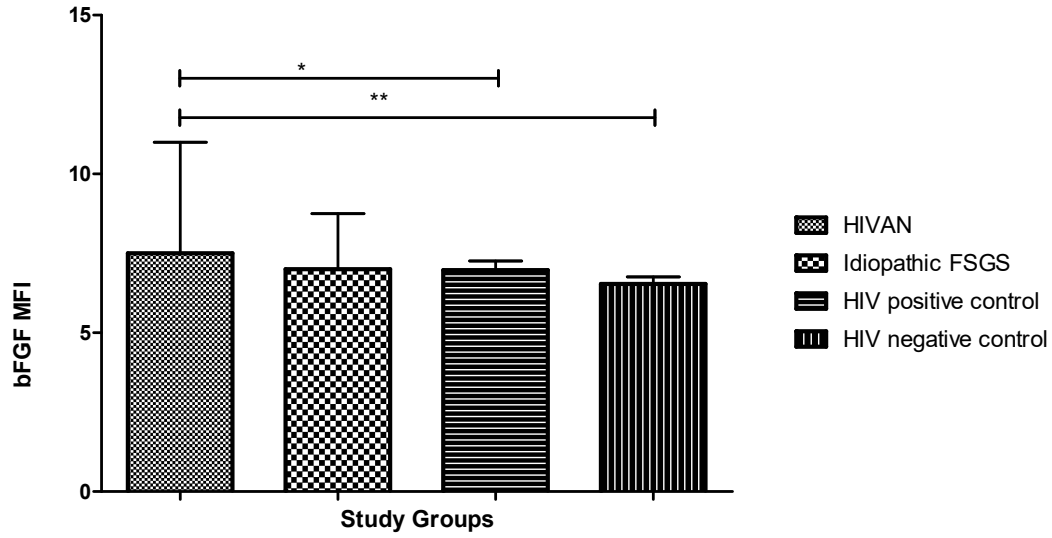


Figure 1: The FMI (median and interquartile) of bFGF in HIVAN, idiopathic FSGS, HIV positive and HIV negative control groups. Serum MFI of bFGF was statistically different between the study groups, $p = 0.003$.

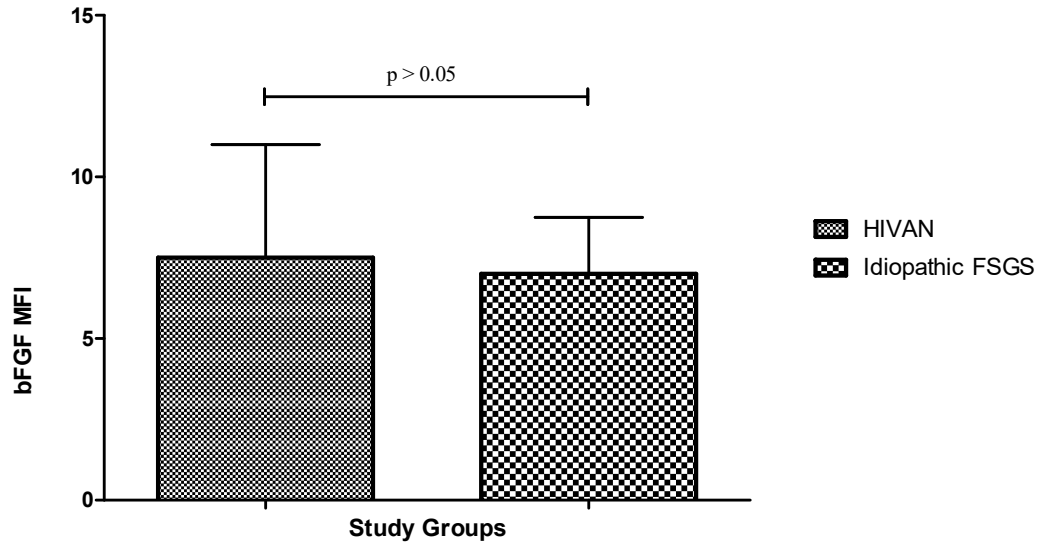


Figure 2: Serum MFI between HIVAN and idiopathic FSGS groups. Result are presented as median and interquartile range. Serum MFI between the two groups are not significantly different, $p = 0.068$.

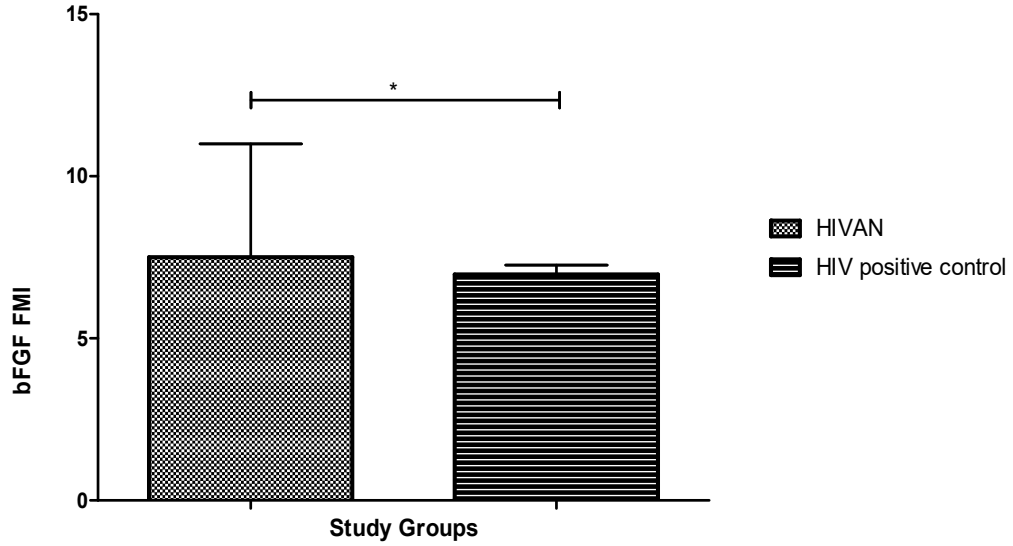


Figure 3: Serum MFI between HIVAN and HIV positive control group. Result are presented as median and interquartile range. Serum MFI between the two groups was significantly different, $p = 0.004$.

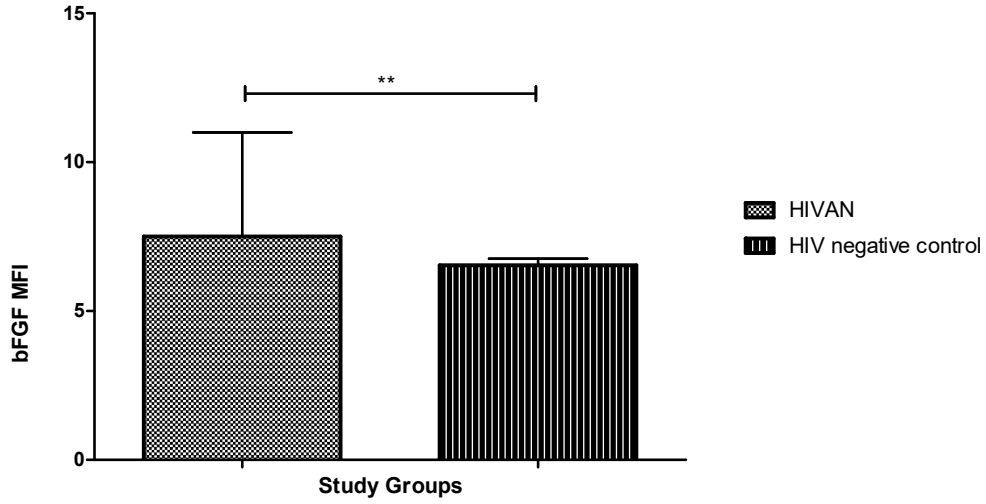


Figure 4: Serum MFI between HIVAN and HIV negative control group. Result are presented as median and interquartile range. Serum MFI between the two groups was significantly different, p = 0.028.

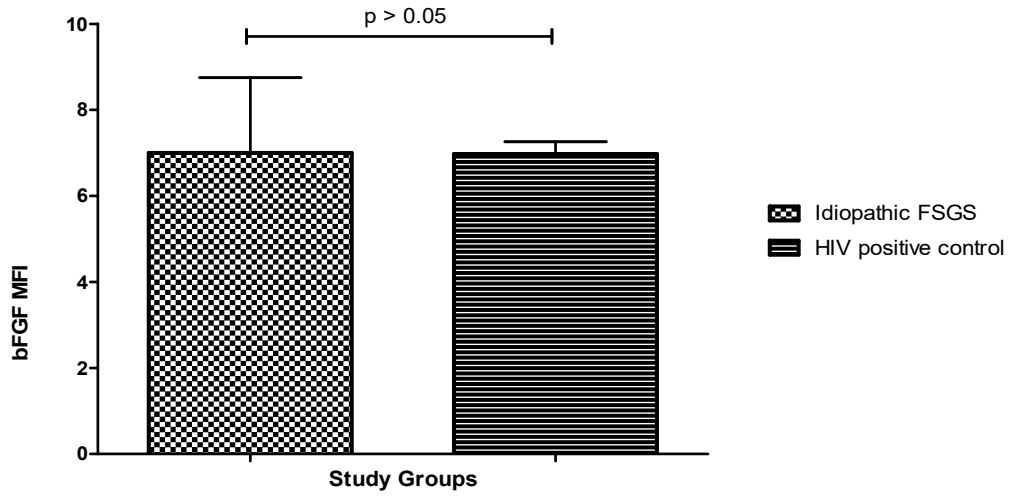


Figure 5: Serum MFI between Idiopathic FSGS and HIV positive control group. Result are presented as median and interquartile range. Serum MFI between the Idiopathic FSGS and HIV positive group was observed to be not significantly different, p = 0.702.

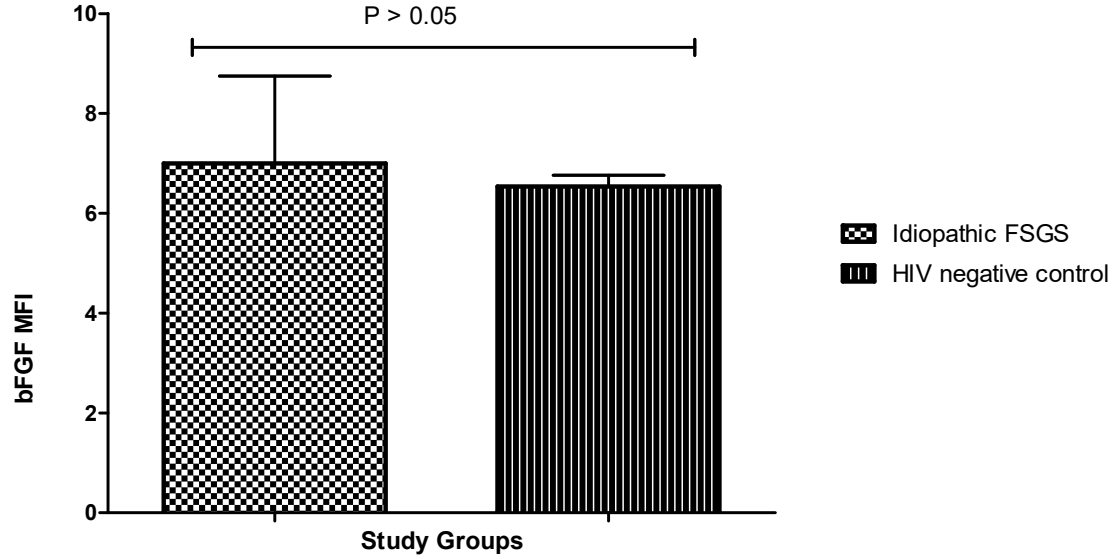


Figure 6: Serum MFI between Idiopathic FSGS and HIV negative control group. Result are presented as median and interquartile range. No statistical significant was observed between the two groups, p = 0.181.

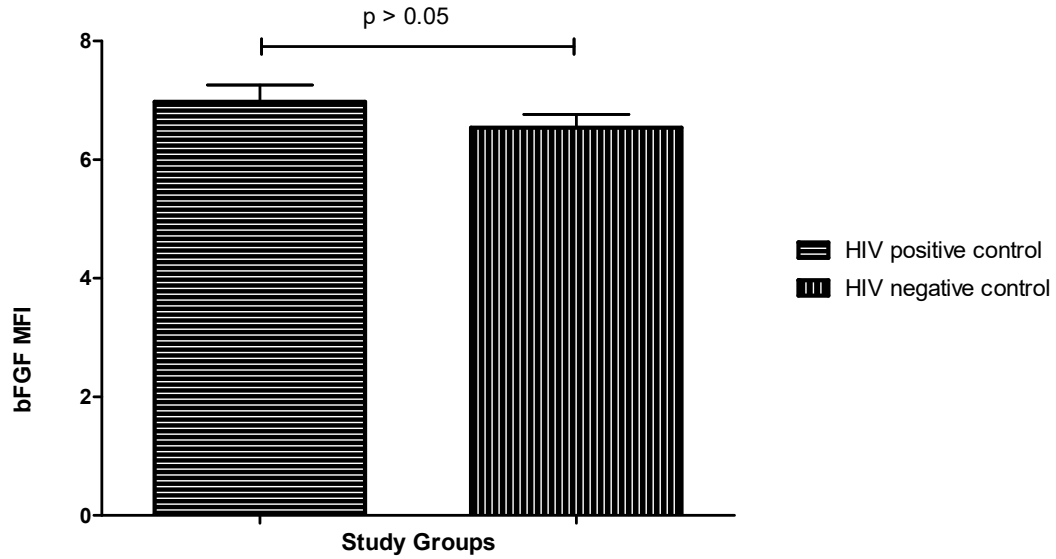


Figure 7: Serum MFI between HIV positive and HIV negative children control groups. Result are presented as median and interquartile range. Serum MFI are not significantly different between the HIV positive and HIV negative control groups, p = 0.318.

Table 1: Clinical and laboratory demographics of patients, expressed as mean ± standard deviation

	HIVAN n = 14	Primary FSGS n = 20	HIV Positive Control n = 20	HIV Negative Control n = 20
Age (years)	10 ± 3.62	9 ± 3.11	11 ± 3.25	7 ± 3.87
Weight (kg)	31.13 ± 11.57	29.04 ± 10.33	37.25 ± 13.73	18.20 ± 10.55
Creatinine (µ/mol)	82.83 ± 62.36	45.32 ± 15.44	40.10 ± 9.25	29.14 ± 9.25
Protein:creatinine ratio	3.07 ± 2.04	4.22 ± 4.21	-	1.85 ± 0.67
eGFR (ml/ min /1.73m^2)	119.98±57.84	125.09±102.53	207.13±58.02	218.00±122.01
Urea blood (mmol/l)	5.30 ± 4.26	7.64 ± 7.02	5.34 ± 9.81	2.71 ± 1.58
Albumin (g/l)	29.91 ± 14.76	28.96 ± 7.96	30.77 ± 10.94	37.30 ± 9.25
Cholesterol (mmol/l)	4.82 ± 2.36	8.21 ± 4.80	3.75 ± 0.68	-
CD4 count	820.20±642.10		900.60±620.00	

HIVAN - Human immunodeficiency virus associated nephropathy

FSGS - Focal segmental glomerulosclerosis

CHAPTER THREE

SYNTHESIS

Human Immunodefiency Virus (HIV) is a disease that raises serious concerns nationwide, with a prevalence of about 8% (Olawumi and Olatunji, 2006). In HIV infected patients, nephropathy is a common outcome (Röling *et al*., 2006). Direct influence of HIV has a substantial role in the development of HIV-associated nephropathy (HIVAN) (Röling *et al*., 2006). HIVAN is commonly related with rapid progression to end-stage kidney disease (ESKD), occurring in persons who are newly diagnosed with advanced HIV infection (Wyatt *et al*., 2007). It has a discrete histology demonstrating a collapsing arrangement of focal segmental glomerulosclerosis (FSGS) (Wyatt *et al*., 2007). HIVAN pathogenesis involves local HIV infection of the kidney, with the virus causing injury to the glomerular and tubular epithelial cells (Wyatt *et al*., 2007). FSGS accounts for nearly 20% of incidence of the nephrotic syndrome in children, with Black children showing a unique susceptibility (D'Agati *et al*., 2011).

Kidney biopsy is the only conclusive approach of establishing a diagnosis (Soler-García *et al.*, 2009), but given the complication of performing this procedure in HIV-infected children, biomarkers should be investigated. Basic fibroblast growth factor (bFGF) is a growth factor and signaling protein encoded by the *FGF2* gene (Dionne *et al*., 1990). It is an angiogenic growth factor that participates in kidney injury and in the pathogenesis of kidney disease (Liu *et al*., 2001).

This study describes the role of bFGF as a potential biomarker in the detection of FSGS in HIV positive and negative children. We noted high levels of bFGF in children with HIVAN compared to HIV negative and HIV positive controls (with no kidney disease). These findings when compared with previously published studies of HIVAN in children suggest that bFGF may have a significant clinical role for the detection of FSGS in HIV infected children. Current studies have displayed the crucial role of biologically active bFGF in the

pathogenesis and development of kidney disease. *Liu et al.,* found high levels of bFGF in the kidney and circulation of HIV-transgenic mice; HIV infected children with renal disease (Liu et al., 2001), which was also demonstrated in our study. These findings suggest that bFGF is released into the systemic circulation and renal interstitium by injured endothelial and renal epithelial cells, and that it may play a role in the pathogenesis of hemolytic-uremic syndrome and HIV nephropathy (Liu *et al*., 2001).

The present study observed an increase of bFGF in HIVAN affected children in contrast to the levels of bFGF in controls. In healthy persons, bFGF is identified in glomerular Bowman's capsule and the wall of blood vessels, with minimal staining covering the renal extracellular matrix (Ray *et al*., 1999). Over the years, several studies have supported this notion such as the one done by *Soler-Garcia et al* that showed levels of bFGF were higher in patients with HIVAN in contrast to those without kidney disease (Soler-García *et al*., 2009). Furthermore, comparable outcomes were observed in HIV-Tg 26 mice correlating to these findings (Soler-García *et al*., 2009). Nonetheless, in HIVAN kidneys, bFGF was excessively high in the renal glomeruli (Soler-García *et al*., 2009). *Rall et al* discovered high circulating and renal tissue levels of bFGF in children suffering from acute stages of renal disease (Rall *et al*., 1985). Consequently, these findings further upkeeps the theory that bFGF released by injured glomerular endothelial cells amass in the HIVAN kidneys leading to renal cell death. Despite the knowledge that has been accumulated, up until now there is still insufficient data to support how bFGF becomes solubilized and hence activated in the human kidney (Liu *et al*., 2001). There is a possibility that bFGF solubilisation occurs from the digestion of the glycosaminoglycan portion of the cells attachment molecule by heparanases, released by blood monoclear cells which in turn activate bFGF (Moscatelli, 1992). Another possibility could be digestion of the protein backbone HPSG by proteases thus releasing bFGF from the immobilized state resulting to its activation (Yayon *et al*., 1991). However, at present it is

still unclear to what extent proteases and heparanases exert for the release of bFGF in the kidney (Liu *et al*., 2001). bFGF is expressed more in children kidneys than in adults; vascular lesions are a crucial part of the immunodeficiency syndrome pathogenesis, even if HIVAN is not present (Ray *et al.*, 1999). Therefore bFGF is possibly released during this process resulting in substantial levels of bFGF in HIV infected children.

In vitro studies done in renal tubular epithelial cells collected from HIVAN children demonstrated that not a lot of colonies are positive for HIV-1, these findings strongly support that other mitogenic factors acting in relation with HIV-1 should be accountable for enhancing the tubule-intestitial proliferative lesions characteristic of HIVAN (Ray, 2009). For instance, renal tubular epithelial cells collected from children with HIVAN accumulate and release substantial amount of heparin-binding growth factors such as FGF-binding protein (BP-) that increase the release and mitogenic activity of some FGF family members (Ray *et al*., 2006). Since high levels of bFGF have been associated with HIVAN in children, bFGF released into the circulation could be confined in damaged renal cells attached to renal heparin sulphate proteoglycans (HSPG) (Ray, 2009). In this way, bFGF can enhance the binding of HIV infected monoclear cells to renal epithelial cells, increase the hypoxia-inducible gene expression in these cells, and enhance renal microcysts and lead to HIVAN development (Ray, 2009). Moreover, HPSG has the ability to function as low-affinity receptors for the accumulation of HIV-1 including other heparin binding factors (Roderiquez *et al*., 1995). In support of this hypothesis, HIVAN children and HIV-Tg26 mice with kidney failure demonstrate a significant renal HSPG up-regulation which correlates with their kidney disease progression and the enhancement of heparin-binding growth factors (Ray *et al*., 2004). Hence, the substantial release and up-regulated level of bFGF in HIVAN children may enhance tubular proliferative lesions as well as the aberrant renal tubular regenerative response (Ray, 2009).

In this study, all HIV infected children with the kidney disease were undergoing antiretroviral therapy hence their CD4 counts, albumin and creatinine levels were well stabilized despite a slightly reduced eGFR compared to children with idiopathic FSGS or controls. These results suggest that antiretroviral therapy preserves kidney function in HIV infected and HIVAN patients. Another study found significant positive correlations between albumin and CD4 count (Olawumi and Olatunji, 2006). (Olawumi and Olatunji, 2006). Albumin has also demonstrated to be a good index of severity of disease because albumin correlates well with CD4 cell count which are traditional indices of severity (Olawumi and Olatunji, 2006). Furthermore, studies in developed countries have displayed consistent results of mean levels of albumin correlating with decreasing of CD4 cell count (Shah *et al*., 2007).

Even though the new anti-retroviral therapies have improved the health of HIV infected children, it has still not been established how they will influence the prevalence and outcome of HIVAN (Ray *et al.*, 2004). The most effective treatment for both HIV-infected patients with chronic kidney disease is HAART. HAART has been found to delay progression to ESKD in African American patients by 38% (Röling *et al*., 2006). Even though there is strong supportive data of HAART as an effective therapy for HIVAN, no controlled, prospective, randomized trials are available to support a positive influence of HAART on the outcome of HIVAN (Röling *et al*., 2006).

In summary, diagnosis of HIVAN requires performing a kidney biopsy, an invasive procedure, that has associated risks in HIV infected children. Also kidney biopsy represents only a small portion of the kidney which may not accurately represent the disease if it is is not homogenously spread throughout the kidney. Therefore, the need for non-invasive biomarkers as a complementary test in the diagnosis of glomerular diseases such as FSGS is important, particularly when renal biopsy is problematic and/or contra-indicated. Thus

biomarkers would be a valuable minimally invasive tool for assessing kidney disease as well as for clinical monitoring of response to antiretroviral therapy in HIVAN.

Conclusion

This study demonstrated statistically significant difference between bFGF levels in children with HIVAN and HIV positive and negative controls, although it failed to distinguish between HIVAN and idiopathic FSGS. Our results in part propose that bFGF is associated with renal injury and that continual use of HAART may slow progression to ESRD. However, further studies with a larger sample size are required to assess bFGF as a potential predictive tool and its prognostic role in HIVAN development.

CHAPTER FOUR

References

AGARWAL, R. Effects of statins on renal function. Mayo Clinic Proceedings, 2007. Elsevier, 1381 1390.

ALLISON, A. C. & EUGUI, E. M. 2000. Mycophenolate mofetil and its mechanisms of action. *Immunopharmacology,* 47**,** 85-118.

ATTA, M. G., GALLANT, J. E., RAHMAN, M. H., NAGAJOTHI, N., RACUSEN, L. C., SCHEEL, P. J. & FINE, D. M. 2006. Antiretroviral therapy in the treatment of HIV-associated nephropathy. *Nephrology Dialysis Transplantation,* 21**,** 2809-2813.

BARISONI, L., SCHNAPER, H. W. & KOPP, J. B. 2009. Advances in the biology and genetics of the podocytopathies: implications for diagnosis and therapy. *Archives of pathology & laboratory medicine,* 133**,** 201-216.

BHIMMA, R., PURSWANI, M. U. & KALA, U. 2013. Kidney disease in children and adolescents with perinatal HIV-1 infection. *Journal of the International AIDS Society,* 16.

BROOKS, A. N., KILGOUR, E. & SMITH, P. D. 2012. Molecular pathways: fibroblast growth factor signaling: a new therapeutic opportunity in cancer. *Clinical cancer research,* 18**,** 1855-1862.

BURGESS, W. H. & MACIAG, T. 1989. The heparin-binding (fibroblast) growth factor family of proteins. *Annual review of biochemistry,* 58**,** 575-602.

CARIDI, G., GIGANTE, M., RAVANI, P., TRIVELLI, A., BARBANO, G., SCOLARI, F., DAGNINO, M., MURER, L., MURTAS, C. & EDEFONTI, A. 2009. Clinical features and long-term outcome of nephrotic syndrome associated with heterozygous NPHS1 and NPHS2 mutations. *Clinical Journal of the American Society of Nephrology,* 4**,** 1065-1072.

CARIDI, G., PERFUMO, F. & GHIGGERI, G. M. 2005. NPHS 2(Podocin) Mutations in Nephrotic Syndrome. Clinical Spectrum and Fine Mechanisms. *Pediatric research,* 57**,** 54R.

CHEN, Y. M. & LIAPIS, H. 2015. Focal segmental glomerulosclerosis: molecular genetics and targeted therapies. *BMC nephrology,* 16**,** 101.

CORESH, J., BYRD-HOLT, D., ASTOR, B. C., BRIGGS, J. P., EGGERS, P. W., LACHER, D. A. & HOSTETTER, T. H. 2005. Chronic kidney disease awareness, prevalence, and trends among US adults, 1999 to 2000. *Journal of the American Society of Nephrology,* 16**,** 180-188.

D'AGATI, V. Pathologic classification of focal segmental glomerulosclerosis. Seminars in nephrology, 2003. Elsevier, 117-134.

D'AGATI, V. D., KASKEL, F. J. & FALK, R. J. 2011. Focal segmental glomerulosclerosis. *New England Journal of Medicine,* 365**,** 2398-2411.

DIONNE, C., CRUMLEY, G., BELLOT, F., KAPLOW, J., SEARFOSS, G., RUTA, M., BURGESS, W., JAYE, M. & SCHLESSINGER, J. 1990. Cloning and expression of two distinct high-affinity receptors cross-reacting with acidic and basic fibroblast growth factors. *The EMBO Journal,* 9**,** 2685-2692.

DUDLEY, A. T., LYONS, K. M. & ROBERTSON, E. J. 1995. A requirement for bone morphogenetic protein-7 during development of the mammalian kidney and eye. *Genes & development,* 9**,** 2795-2807.

FOGO, A. B. 2015. Causes and pathogenesis of focal segmental glomerulosclerosis. *Nature Reviews Nephrology,* 11**,** 76.

FREDRICK, F., FRANCIS, J. M., RUGGAJO, P. J. & MARO, E. E. 2016. Renal abnormalitiesamong HIV infected children at Muhimbili National Hospital (MNH)—Dar es Salaam, Tanzania. BMC nephrology, 17, 30.

HUSAIN, N. E., AHMED, M. H., ALMOBARAK, A. O., NOOR, S. K., ELMADHOUN, W. M., AWADALLA, H., WOODWARD, C. L. & MITAL, D. 2018. HIV-Associated Nephropathy in Africa: Pathology, Clinical Presentation and Strategy for Prevention. *Journal of clinical medicine research,* 10**,** 1.

ITOH, N., OHTA, H. & KONISHI, M. 2015. Endocrine FGFs: evolution, physiology, pathophysiology, and pharmacotherapy. *Frontiers in endocrinology,* 6**,** 154.

JENNETTE, J. C. & HIPP, C. G. 1985. Clq nephropathy: A distinct pathologic entity usually causing nephrotic syndrome. *American Journal of Kidney Diseases,* 6**,** 103-110.

KALAYJIAN, R. C. 2010. The treatment of HIV-associated nephropathy. *Advances in chronic kidney disease,* 17**,** 59-71.

KARAVANOVA, I. D., DOVE, L. F., RESAU, J. H. & PERANTONI, A. O. 1996. Conditioned medium from a rat ureteric bud cell line in combination with bFGF induces complete differentiation of isolated metanephric mesenchyme. *Development,* 122**,** 4159-4167.

KAUFMAN, L., POTLA, U., COLEMAN, S., DIKIY, S., HATA, Y., KURIHARA, H., HE, J. C., D'AGATI, V. D. & KLOTMAN, P. E. 2010. Up-regulation of the homophilic adhesion molecule sidekick-1 in podocytes contributes to glomerulosclerosis. *Journal of Biological Chemistry,* 285**,** 25677-25685.

KIFFEL, J., RAHIMZADA, Y. & TRACHTMAN, H. 2011. Focal segmental glomerulosclerosis and chronic kidney disease in pediatric patients. *Advances in chronic kidney disease,* 18**,** 332-338.

KIM, H.-S. 1998. Assignment1 of the human basic fibroblast growth factor gene FGF2 to chromosome 4 band q26 by radiation hybrid mapping. *Cytogenetic and Genome Research,* 83**,** 73-73.

KIM, J. S., HAN, B. G., CHOI, S. O. & CHA, S.-K. 2016. Secondary focal segmental glomerulosclerosis: from podocyte injury to glomerulosclerosis. *BioMed research international,* 2016.

KLAGSBRUN, M. & BAIRD, A. 1991. A dual receptor system is required for basic fibroblast growth factor activity. *Cell,* 67**,** 229-231.

KLAHR, S. & MORRISSEY, J. J. 2000. The role of vasoactive compounds, growth factors and cytokines in the progression of renal disease. *Kidney International,* 57**,** S7-S14.

KORBET, S. M. 2000. The treatment of primary focal segmental glomerulosclerosis. *Renal failure,* 22**,** 685-696.

KOPP, J. B. 2000. BMP receptors in kidney. *Kidney international,* 58**,** 2237-2238.

KOPP, J. B., SMITH, M. W., NELSON, G. W., JOHNSON, R. C., FREEDMAN, B. I., BOWDEN, D. W., OLEKSYK, T., MCKENZIE, L. M., KAJIYAMA, H. & AHUJA, T. S. 2008. MYH9 is a major-effect risk gene for focal segmental glomerulosclerosis. *Nature genetics,* 40**,** 1175.

KRIZ, W., HÄHNEL, B., RÖSENER, S. & ELGER, M. 1995. Long-term treatment of rats with FGF-2 results in focal segmental glomerulosclerosis. *Kidney international,* 48**,** 1435-1450.

LEAF, D. E., APPEL, G. B. & RADHAKRISHNAN, J. 2010. Glomerular disease: why is there a dearth of high quality clinical trials? *Kidney international,* 78**,** 337-342.

LI, Z., JEREBTSOVA, M., LIU, X.-H., TANG, P. & RAY, P. E. 2006. Novel cystogenic role of basic fibroblast growth factor in developing rodent kidneys. *American Journal of Physiology-Renal Physiology,* 291**,** F289-F296.

LIU, X.-H., AIGNER, A., WELLSTEIN, A. & RAY, P. E. 2001. Up-regulation of a fibroblast growth factor binding protein in children with renal diseases. *Kidney international,* 59**,** 1717-1728.

LU, T.-C. & ROSS, M. 2005. HIV-associated nephropathy: a brief review. *The Mount Sinai journal of medicine, New York,* 72**,** 193-199.

MARKOWITZ, G. S., SCHWIMMER, J. A., STOKES, M. B., NASR, S., SEIGLE, R. L., VALERI, A. M. & D'AGATI, V. D. 2003. C1q nephropathy: a variant of focal segmental glomerulosclerosis. *Kidney international,* 64**,** 1232-1240.

MENDOZA, S. A., REZNIK, V. M., GRISWOLD, W. R., KRENSKY, A. M., YORGIN, P. D. & TUNE, B. M. 1990. Treatment of steroid-resistant focal segmental glomerulosclerosis with pulse methylprednisolone and alkylating agents. *Pediatric nephrology,* 4**,** 303-307.

MERCIER, K., SMITH, H. & BIEDERMAN, J. 2014. Renin-angiotensin-aldosterone system inhibition: overview of the therapeutic use of angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, mineralocorticoid receptor antagonists, and direct renin inhibitors. *Primary Care: Clinics in Office Practice,* 41**,** 765-778.

MEYRIER, A. Y. 2009. Treatment of focal segmental glomerulosclerosis with immunophilin modulation: when did we stop thinking about pathogenesis? *Kidney international,* 76**,** 487-491.

MOSCATELLI, D. 1992. Basic fibroblast growth factor (bFGF) dissociates rapidly from heparan sulfates but slowly from receptors. Implications for mechanisms of bFGF release from pericellular matrix. *Journal of Biological Chemistry,* 267**,** 25803-25809.

MULLINS, L. J., CONWAY, B. R., MENZIES, R. I., DENBY, L. & MULLINS, J. J. 2016. Renal disease pathophysiology and treatment: contributions from the rat. *Disease models & mechanisms,* 9**,** 1419-1433.

NUSAYR, E., SADIDEEN, D. T. & DOETSCHMAN, T. 2013. FGF2 modulates cardiac remodeling in an isoform-and sex-specific manner. *Physiological reports,* 1.

OELLERICH, M., SHIPKOVA, M., SCHÜTZ, E., WIELAND, E., WEBER, L., TÖNSHOFF, B., ARMSTRONG, V. W. & RECIPIENTS, G. S. G. O. M. M. T. I. P. R. T. 2000. Pharmacokinetic and metabolic investigations of mycophenolic acid in pediatric patients after renal transplantation: implications for therapeutic drug monitoring. *Therapeutic drug monitoring,* 22**,** 20-26.

OKPECHI, I. G., AMEH, O. I., BELLO, A. K., RONCO, P., SWANEPOEL, C. R. & KENGNE, A. P. 2016. Epidemiology of histologically proven glomerulonephritis in Africa: a systematic review and meta-analysis. *PloS one,* 11**,** e0152203.

OLAWUMI, H. & OLATUNJI, P. 2006. The value of serum albumin in pretreatment assessment and monitoring of therapy in HIV/AIDS patients. *HIV medicine,* 7**,** 351-355.

OLSEN, S. K., GARBI, M., ZAMPIERI, N., ELISEENKOVA, A. V., ORNITZ, D. M., GOLDFARB, M. & MOHAMMADI, M. 2003. Fibroblast growth factor (FGF) homologous factors share structural but not functional homology with FGFs. *Journal of Biological Chemistry,* 278**,** 34226-34236.

ORNITZ, D. M. & ITOH, N. 2015. The fibroblast growth factor signaling pathway. *Wiley Interdisciplinary Reviews: Developmental Biology,* 4**,** 215-266.

ORNITZ, D. M. & ITOH, N. 2016. An Introduction to the Fibroblast Growth Factors. *Fibroblast Growth Factors: Biology and Clinical Application: FGF Biology and Therapeutics***,** 1.

ORNITZ, D. M., XU, J., COLVIN, J. S., MCEWEN, D. G., MACARTHUR, C. A., COULIER, F., GAO, G. & GOLDFARB, M. 1996. Receptor specificity of the fibroblast growth factor family. *Journal of Biological Chemistry,* 271**,** 15292-15297.

PHILIPPE, A., NEVO, F., ESQUIVEL, E. L., REKLAITYTE, D., GRIBOUVAL, O., TÊTE, M.-J., LOIRAT, C., DANTAL, J., FISCHBACH, M. & POUTEIL-NOBLE, C. 2008. Nephrin mutations can cause childhood-onset steroid-resistant nephrotic syndrome. *Journal of the American Society of Nephrology,* 19**,** 1871-1878.

RALL, L. B., SCOTT, J., BELL, G. I., CRAWFORD, R. J., PENSCHOW, J. D., NIALL, H. D. & COGHLAN, J. P. 1985. Mouse prepro-epidermal growth factor synthesis by the kidney and other tissues. *Nature,* 313**,** 228.

RAMSURAN, D., BHIMMA, R., RAMDIAL, P. K., NAICKER, E., ADHIKARI, M., DEONARAIN, J., SING, Y. & NAICKER, T. 2012. The spectrum of HIV-related nephropathy in children. *Pediatric nephrology,* 27**,** 821-827.

RAY, P. E. 2009. Taking a hard look at the pathogenesis of childhood HIV-associated nephropathy. *Pediatric nephrology,* 24**,** 2109-2119.

RAY, P. E., LIU, X.-H., XU, L. & RAKUSAN, T. 1999. Basic fibroblast growth factor in HIV-associated hemolytic uremic syndrome. *Pediatric Nephrology,* 13**,** 586-593.

RAY, P. E., TASSI, E., LIU, X.-H. & WELLSTEIN, A. 2006. Role of fibroblast growth factor-binding protein in the pathogenesis of HIV-associated hemolytic uremic syndrome. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology,* 290**,** R105-R113.

RAY, P. E., XU, L., RAKUSAN, T. & LIU, X.-H. 2004. A 20-year history of childhood HIV-associated nephropathy. *Pediatric nephrology,* 19**,** 1075-1092.

RODERIQUEZ, G., ORAVECZ, T., YANAGISHITA, M., BOU-HABIB, D. C., MOSTOWSKI, H. & NORCROSS, M. A. 1995. Mediation of human immunodeficiency virus type 1 binding by interaction of cell surface heparan sulfate proteoglycans with the V3 region of envelope gp120-gp41. *Journal of Virology,* 69**,** 2233-2239.

RÖLING, J., SCHMID, H., FISCHEREDER, M., DRAENERT, R. & GOEBEL, F. 2006. HIV-Associated Renal Diseases and Highly Active Antiretroviral Therapy—Induced Nephropathy. *Clinical Infectious Diseases,* 42**,** 1488-1495.

ROOD, I. M., DEEGENS, J. K. & WETZELS, J. F. 2012. Genetic causes of focal segmental glomerulosclerosis: implications for clinical practice. Oxford University Press.

ROY, S. & STAPLETON, F. B. 1987. Focal segmental glomerulosclerosis in children: comparison of nonedematous and edematous patients. *Pediatric Nephrology,* 1**,** 281-285.

SCHREIBER, A. B., KENNEY, J., KOWALSKI, W. J., FRIESEL, R., MEHLMAN, T. & MACIAG, T. 1985. Interaction of endothelial cell growth factor with heparin: characterization by receptor and antibody recognition. *Proceedings of the National Academy of Sciences,* 82**,** 6138-6142.

SCHWARTZ, G. J., MUNOZ, A., SCHNEIDER, M. F., MAK, R. H., KASKEL, F., WARADY, B. A. & FURTH, S. L. 2009. New equations to estimate GFR in children with CKD. *Journal of the American Society of Nephrology,* 20**,** 629-637.

SHAH, S., SMITH, C., LAMPE, F., YOULE, M., JOHNSON, M., PHILLIPS, A. & SABIN, C. 2007. Haemoglobin and albumin as markers of HIV disease progression in the highly active antiretrovial therapy era: relationships with gender. *HIV medicine,* 8**,** 38-45.

SHEPHERD, J., COBBE, S. M., FORD, I., ISLES, C. G., LORIMER, A. R., MACFARLANE, P. W., MCKILLOP, J. H. & PACKARD, C. J. 1995. Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. *New England Journal of Medicine,* 333**,** 1301-1308.

SOLER-GARCÍA, Á. A., RAKHMANINA, N. Y., MATTISON, P. C. & RAY, P. E. 2009. A urinary biomarker profile for children with HIV-associated renal diseases. *Kidney international,* 76**,** 207-214.

SONI, S. S., RONCO, C., KATZ, N. & CRUZ, D. N. 2009. Early diagnosis of acute kidney injury: the promise of novel biomarkers. *Blood purification,* 28**,** 165-174.

STOKES, M., VALERI, A., MARKOWITZ, G. & D'AGATI, V. 2006. Cellular focal segmental glomerulosclerosis: clinical and pathologic features. *Kidney international,* 70**,** 1783-1792.

STRUTZ, F., ZEISBERG, M., HEMMERLEIN, B., SATTLER, B., HUMMEL, K., BECKER, V. & MÜLLER, G. A. 2000. Basic fibroblast growth factor expression is increased in human renal fibrogenesis and may mediate autocrine fibroblast proliferation. *Kidney international,* 57**,** 1521-1538.

TANG, P., JEREBTSOVA, M., PRZYGODZKI, R. & RAY, P. E. 2005. Fibroblast growth factor-2 increases the renal recruitment and attachment of HIV-infected mononuclear cells to renal tubular epithelial cells. *Pediatric Nephrology,* 20**,** 1708-1716.

VILLANUEVA, S., CESPEDES, C., GONZALEZ, A. A., ROESSLER, E. & VIO, C. P. 2008. Inhibition of bFGF-receptor type 2 increases kidney damage and suppresses nephrogenic protein expression after ischemic acute renal failure. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology,* 294**,** R819-R828.

VILLANUEVA, S., CONTRERAS, F., TAPIA, A., CARREÑO, J. E., VERGARA, C., EWERTZ, E., CESPEDES, C., IRARRAZABAL, C., SANDOVAL, M. & VELARDE, V. 2013. Basic fibroblast growth factor reduces functional and structural damage in chronic kidney disease. *American Journal of Physiology-Renal Physiology,* 306**,** F430-F441.

VILLANUEVA, S., CONTRERAS, F., TAPIA, A., CARREÑO, J. E., VERGARA, C., EWERTZ, E., CESPEDES, C., IRARRAZABAL, C., SANDOVAL, M. & VELARDE, V. 2014. Basic fibroblast growth factor reduces functional and structural damage in chronic kidney disease. *American Journal of Physiology-Renal Physiology,* 306**,** F430-F441.

VIRAG, J. A., ROLLE, M. L., REECE, J., HARDOUIN, S., FEIGL, E. O. & MURRY, C. E. 2007. Fibroblast growth factor-2 regulates myocardial infarct repair: effects on cell proliferation, scar contraction, and ventricular function. *The American journal of pathology,* 171**,** 1431-1440.

WHARRAM, B. L., GOYAL, M., WIGGINS, J. E., SANDEN, S. K., HUSSAIN, S., FILIPIAK, W. E., SAUNDERS, T. L., DYSKO, R. C., KOHNO, K. & HOLZMAN, L. B. 2005. Podocyte depletion causes glomerulosclerosis: Diphtheria toxin–induced podocyte depletion in rats expressing human diphtheria toxin receptor transgene. *Journal of the American Society of Nephrology,* 16**,** 2941-2952.

WOLF, G., BUTZMANN, U. & WENZEL, U. O. 2003. The renin-angiotensin system and progression of renal disease: from hemodynamics to cell biology. *Nephron physiology,* 93**,** p3-p13.

WYATT, C. M., WINSTON, J. A., MALVESTUTTO, C. D., FISHBEIN, D. A., BARASH, I., COHEN, A. J., KLOTMAN, M. E. & KLOTMAN, P. E. 2007. Chronic kidney disease in HIV infection: an urban epidemic. *Aids,* 21**,** 2101-2103.

WYATT, C. M. & KLOTMAN, P. E. 2009. HIV-associated nephropathy. *Genetic Diseases of the Kidney.* Elsevier.

WYATT, C. M. 2017. Kidney disease and HIV infection. *Topics in antiviral medicine,* 25**,** 13.

YAYON, A., KLAGSBRUN, M., ESKO, J. D., LEDER, P. & ORNITZ, D. M. 1991. Cell surface, heparin-like molecules are required for binding of basic fibroblast growth factor to its high affinity receptor. *Cell,* 64**,** 841-848.