UNIVERSITY OF GHANA

COLLEGE OF HEALTH SCIENCES

SERUM PROTEINS (ALPHA, BETA, AND GAMMA GLOBULINS) AND ONCOTIC PRESSURE IN GHANAIAN PATIENTS WITH NEPHROTIC SYNDROME

BY

EGYAM BILL CLINTON

(10636541)

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DECLARATION

I, Egyam Bill Clinton, declare that with the exception of research by other individuals, which have been duly acknowledged by citation and referencing, the work presented in this thesis is the result of my own research carried out at the laboratories of MDS-Lancet Laboraroties Ghana Limited, under the supervision of Dr. Seth Amanquah and Dr. Emmanuel Ofori, both of the Department of Chemical Pathology, School of Biomedical and Allied Health Sciences, College of Health Sciences, University of Ghana, and has not been submitted to any other institution for the award of any other degree.

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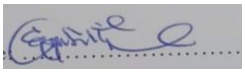
Signature: …  Date: …15Th APRIL 2021…

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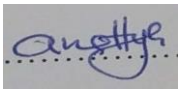
Signature: …  Date: …15/04/2021…

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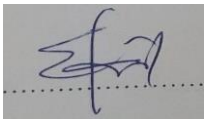
Signature:  Date: …15/04/2021….

Dr. Emmanuel Ofori

DEDICATION

To my supportive family

ACKNOWLEDGEMENT

I am deeply indebted to the following individuals who have contributed, in diverse ways, to the success of this research: my supervisors (Drs. Seth Amanquah and Emmanuel Ofori), the management and staff of MDS-Lancet Laboraroties Ghana Limited, colleague MPhil Chemical Pathology candidates, and my wife, Mrs. Clinton.

ABSTRACT

Background: Nephrotic syndrome, which involves the leakage of serum proteins, especially albumin, into urine, is an important cause of chronic kidney disease (CKD) in sub-Saharan Africa, and hence constitutes a major threat to public health. Nonetheless, current studies on the condition are limited, especially in Africa. Moreover, studies reporting on proteinuria and oncotic pressure in patients with nephrotic syndrome have predominantly focused on the predominant protein lost – albumin. However, to compensate for the absence of albumin, the liver synthesizes other proteins. Yet, little is known about these other proteins, and their clinical relevance is yet to be fully elucidated.

General aim: To investigate oncotic pressure, albumin and non-albumin proteins in the serum of Ghanaian patients with and without nephrotic syndrome

Methodology: This was a case-control study involving ninety-nine (99) individuals with nephrotic syndrome (comprising of 51 males and 48 females) and forty-seven (47) individuals without the disease (comprising of 21 males and 26 females) aged up to 91 years recruited at MDS-Lancet Laboraroties Ghana Limited. Socio-demographic and clinical data of study participants were gathered by means of a standard questionnaire and a review of patients' laboratory request forms. Six milliliters (6 ml) of venous blood sample was taken from each participant, and used to determine albumin, and different globulins in serum, as well as colloid osmotic pressure. Electrophoresis technique was also used to separate proteins, with various fractions determined by a densitometer. The oncotic pressure was calculated using standard factors.

Results: Of the 146 individuals who volunteered as participants of the study, males comprised 51.5% (n = 51) and females comprised 48.5% (n = 48) in the neprotic syndrome group, whereas

in the control group, the males and females comprised 44.7% (n = 21) and 55.3% (n = 26) respectively. The mean age of the study participants was 46.95±22.19 years in the nephrotic syndrome group and 45.72±16.08 years in the control group. Serum levels of alpha-2-globulin, C-reactive protein, urea, gamma globulins, and calcium were significantly higher in the nephrotic syndrome group than in the control group, whereas a decrease was observed for transferrin, total proteins, albumins, beta-1-globulins, and colloid osmotic pressure. Moreover, serum levels of C-reactive protein (OR = 1.41, p = 0.005) and gamma globulin (OR = 4.12, p = 0.005) were independent risk factors, increasing the odds of occurrence of nephrotic syndrome by about one and a half and four folds respectively.

Conclusion: The nephrotic syndrome group were found to have lost C-reactive protein, urea, gamma globulins, and calcium into the serum, co-occurring with a lower colloid osmotic pressure and serum levels of transferrin, total proteins, albumins, and beta-1-globulins compared to the control group. Levels of C-reactive protein and gamma globulin increased the odds of occurrence of nephrotic syndrome.

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LIST OF ABBREVIATIONS

BMI.....Body mass index

CHS.....College of Health Sciences

CKD.....Chronic kidney disease

COP.....Colloid osmotic pressure

EDTA.....Ethylene diamine tetraacetic acid

EPRC.....Ethical and Protocol Review Committee

g.....Gram

GFR.....Glomerular filtration rate

HIV.....Human immunodeficiency virus

Kd.....Kilodalton

Kg.....Kilogram

m.....Metre

ml.....Millilitre

mmHg.....Millimeter mercury

SD.....Standard deviation

SEM.....Standard error of mean

SLE.....Systemic lupus erythematosus

UGMS.....University of Ghana Medical School

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the study

Nephrotic syndrome is a disorder of the kidney that causes the body system to excrete greater amounts of proteins in the urine (Glassock *et al*., 2015). It is typically marked by levels of urinary protein that are above 3.5 g/1.73 m^2 of body-surface area per day (Post & Rose, 2006; Glassock *et al*., 2015). The syndrome occurs due to excessive damage to the cluster of small blood vessels in the glomeruli of the kidney (Glassock *et al*., 2015). Nephrotic syndrome is resultant of troubles with the kidneys' glomeruli, which are are tiny blood vessels in the kidneys responsible for eliminating from the blood wastes and excess fluids, channelling them as urine to the bladder, while selectively permiting the retention of cells and proteins in the blood (Hull & Goldsmith, 2008). In nephrotic syndrome, the compromised glomeruli allow passage of 3 grams or more of proteins into the urine when measured over a twenty four-hour duration, a figure that exceeds that of healthy glomeruli by over twenty folds (Hull & Goldsmith, 2008).

By the body's mechanism, dissolved compounds in the body have an osmotic pressure. This is due to the issue that large plasma proteins cannot easily cross the capillary walls; their effect on the osmotic pressure of the interiors of the capillaries keep in an equilibrium the tendency for capillary fluid leakage. Fluid is pulled into the capillaries by oncotic pressure. Physiologically, where plasma proteins are reduced, especially, being lost in the urine (proteinuria), or from malnutrition, oncotic pressure decreases, and filtration across the

capillary increases, causing a buildup of excess fluid in the tissues (edema) (Lent-Schochet & Jialal, 2020).

It is held that passage of plasma proteins whose sizes are above 70 kd (Kilo Dalton) across the glomerular basement membrane is typically normally restricted by a charge- and a size-selective barrier (Comper *et al*., 1995, Goode *et al*., 1996). The size-selective barrier, which is believed to consist of pores in the glomerular-basement-membrane meshwork, does not permit passage of plasma proteins of bigger sizes (those whose size are greater than 150 kd). However, a damage to this glomerular-basement-membrane meshwork could allow for the leakage of proteins from the blood, especially albumins.

Although nephrotic syndrome could occur at any age, it is more prevalent in children, predominantly, those between ages 1½ and 4 years (Klifa *et al*., 2019). Congenital nephrotic syndromes appear during the first year of life (Kerlin *et al*., 2012). Males aged below eight years old are more affected by the condition than females of the same age, but at ages above eight years, this disparity with respect to gender is less pronounced (Kamei *et al*., 2014). Adults have a 26-fold increased odds of suffering from the condition than children, and women have a two-fold increased odds of suffering from the condition than men (Kerlin *et al*., 2012).

Cardiovascular diseases are the predominant culprits in nephrotic syndrome mortalities by virtue of the pronounced effects the condition exerts on blood (Sethna *et al*., 2018). Hypoalbuminemia, hypercholesterolemia, and hypertriglyceridemia have an impact on nephrotic syndrome and its complications (Semwal, 2020). Additionally, infections and thromboembolic accidents are more common causes of mortality in patients with nephrotic syndrome (Kerlin *et al*., 2012).

1.2 Problem statement

Nephrotic syndrome, which involves the leakage of serum proteins, especially albumin, into urine, is an important cause of chronic kidney disease (CKD) in sub-Saharan Africa (El-Tigani *et al*., 2009; Olowu *et al*., 2013; Asinobi *et al*., 2014; Odetunde *et al*., 2014). Studies in the sub-region have reported its incidence to range between 14.6% and ~50% in relation to its proportion of renal disorders (Hutt & White, 1964; Abdurrahman *et al*., 1990; Eke & Eke, 1994), and 0.35–1.34% as its proportion of hospital admissions (Abdurrahman *et al*., 1990; Okoro *et al*., 2000; Olowu *et al*., 2010a; Olowu *et al*., 2010b).

As a result of the inflammatory condition of the kidney, different proteins are excreted, and the levels of these proteins have largely not been determined in nephrotic syndrome patients in Ghana. The loss of essential proteins that the body needs for adequate metabolism and survival, owing to massive kidney membrane permeability leads to severe edema, with deteriorating complications, such as cardiovascular conditions and coagulopathy (Lent-Schochet & Jialal, 2020). Another major consequence of nephrotic syndrome is the progression to end stage kidney disease, which has been estimated to affect approximately 10.4% of some African populations (Afolabi *et al*., 2009; Sumaili *et al*., 2009). In Ghana, it has been reported at a prevalence of 46.9% among individuals with hypertension, a condition whose course is more aggressive among blacks (Gibbs *et al*., 1999; Osafo *et al*., 2011). Of concern, CKD is independently associated with a high economic burden. In the United States, a high-income region, whose CKD prevalence is below that of Ghana, for instance, about €23.46 billion was spent on CKD management in the year 2010 alone (Xue *et al*., 2001; Chadban *et al*., 2003;

Coresh *et al*., 2007; Chen *et al*., 2009). As Ghana is a low-income region, and has a healthcare system that is under-resourced, such levels of expenditure on CKD could exert an immense economic burden on families, and eventually result in death of individuals. The poor prognosis of nephrotic syndrome and its resultant CKD, as well as the potential negative economic impact it could have on sufferers of the condition and their families make it a major threat to public health, and warrants the implementation of public health strategies to combat it.

1.3 Justification of the study

Despite the clinical significance of the nephrotic syndrome, current studies on the condition are limited, especially in sub-Saharan Africa. Moreover, studies reporting on proteinuria and oncotic pressure in patients with nephrotic syndrome have predominantly focused on albumin, as it is the predominant protein lost. However, it is noted that in the absence of albumin, the liver is forced to synthesize other proteins necessary for the maintenance of the oncotic pressure, such as alpha-2-macroglobulins and beta lipoproteins. Yet, little is known about these other proteins that could support a balance on the oncotic pressure in the absence of serum albumin, and the role they play in the severity of the disease. In addition, their clinical relevance is yet to be fully elucidated, and this limits the overall management of nephrotic syndrome. In order to contribute to filling these knowledge gaps, and help improve the management of individuals with nephrotic syndrome, this study aimed at investigating oncotic pressure, albumin and non-albumin proteins in the serum of Ghanaian patients with and without nephrotic syndrome.

1.4 General aim

The aim of this study was to investigate oncotic pressure, albumin, and non-albumin proteins in the serum of Ghanaian patients with and without nephrotic syndrome.

1.5 Specific objectives

The specific objectives of the study were:

- To compare the levels of albumin and non-albumin proteins between patients with and without nephrotic syndrome, using quantifiable electrophoretic assay techniques
- To compare colloid osmotic pressure levels between the nephrotic syndrome and control groups
- To determine predictors of nephrotic syndrome among the study participants

1.6 Hypotheses

1.6.1 Null hypothesis

Non-albumin proteins have no influence on oncotic pressure among patients with nephrotic syndrome.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 An overview of nephrotic syndrome

Nephrotic syndrome is characterized by a massive proteinuria (particularly, albuminuria) because of heterogeneous dysfunction of the glomerulus, which has detrimental effects on long-term renal function (Noone *et al*., 2018; Shamna & Shijikumar, 2020).

The protein albumin acts like a sponge, and draws excess fluid from body tissues into the bloodstream, and is kept there until removal by the kidneys. The presence of albumin in urine (albuminuria) leads to a retention in blood albumin because of damage to the glomeruli, resulting in hypovolemia and subsequently, edema (Hull & Goldsmith, 2008). Mechanisms that cause a loss in selectivity of the capillary walls of the glomeruli promote the underlying haematuria and proteinuria of glomerular diseases (AlSahow *et al*., 2019). Proteinuria that is more than 85% albumin is referred to as selective proteinuria (Perico *et al*., 2019). Albumin has a net negative charge, and it is proposed that loss of glomerular membrane negative charges could be important in causing albuminuria. Non-selective proteinuria involving plasma protein leakage is restricted to permeability defects, and does not alter the net charge of the glomeruli. Except in cases of minimal-change nephropathy, in which selective permeability is maintained to a large extent, this construct is limited in its allowance for a clear-cut separation of proteinuria causes (Sinnakirouchenan, 2020). As an example, alpha-2-macroglobulin, which is a large molecular weight protein, does not leak into the urine, and hence its concentration rises by 10-fold or more in the nephrotic syndrome when other lower molecular weight proteins are lost in

the urine (Rehman *et al*., 2013). This relative increase in the serum concentration of alpha-2-macroglobulin is detrimental to the maintenance of oncotic pressure (Rehman *et al*., 2013). However, no specific deficiency or disease state has been attributed to low concentrations of alpha-2-macroglobulin (Rehman *et al*., 2013).

The quantities of albumins generate a significant proportion of capillary oncotic pressure – the protein makes up about four-fifths of the total blood plasma-exerted oncotic pressure (28 mmHg) on interstitial fluid (Semwal, 2020). The capillary osmotic pressure acts as a balance that pulls water into the vessels, given that the proteins in blood are unable to pass out via capillary endothelium (Guyton *et al*., 2006).

Neprotic syndrome has basically been categorized into two – primary and secondary. The syndrome is said to be primary when the damage is exclusively to the kidneys, and secondary when other body parts are also involved (Brunkhorst, 2014). The syndrome has also been categorized based on steroid responsiveness – steroid-sensitive and -resistant (Lee *et al*., 2019). It is said to be steroid-sensitive when glucocorticoid treatment results in remission, and conversely said to be resistant when remission is incomplete after an eight-week corticosteroid therapy (Lee *et al*., 2019).

A significant proportion of the syndrome remains multifaceted and idiopathic, and per a paediatric population of a hundred thousand, the incidence ranges between two and seven cases (MacCloskey & Maxwell, 2017). When the disease burden is categorized by age, adults and children have a somewhat invariably distinct manifestation of the pathology (Canetta & Radhakrishnan, 2015).

Changes in the colloidal osmotic pressure (oncotic pressure) one involves in the pathophysiology of the nephrotic syndrome. Oncotic pressure is an essential determinant of the

intravascular volume (Waddell, 2014). The disrupted capillary hydrostatic pressure-oncotic pressure equilibrium is a critical determinant of liver cirrhosis and edema onset; when oncotic pressure is reduced, contributes significantly to production of ascites among renal sodium retention patients (Waddell, 2014).

2.2 Causes of nephrotic syndrome

Alchi & Jayne (2010) and Hull & Goldsmith (2008) have, on the basis of histological patterns, respectively provided insights on major causes of the two categories of the syndrome – primary and secondary. Alchi & Jayne (2010) placed the major causes under the following subgroupings, providing explanations in each case: rapid progressive, membranoproliferative, and membranous glomerulonephritis, focal segmental glomerulosclerosis, and minimal change disease. The authors noted that in the rapid progressive form, over a short period, such as between a few day and three months, the rate of glomerular filtration drops swiftly by half, at a minimum, and the glomerili are observed to have a crescent shape. As regards the membranoproliferative, filtration through the glomeruli becomes exacting, owing to a composite effect of clogging of the membranes by antibody deposits and glomerular inflammation. With respect to the membranous too, the membranes of the glomeruli become inflamed, causing an increase in leakages in the kidney – the mechanism underlying the inflammation has been hypothesized to involve autoimmunity. In the case of the focal segmental glomerulosclerosis, which frequently occurs in adults, there are apparent scarring of parts of the glomerular tissue, with the damage occurring in only sections of the glomeruli, whereas other parts remain intact. As regards the minimal change disease, it frequently occurs

in children, having severe proteinuria as one of its symptoms, and the damage to the nephrons are so inconspicuous that the present lesions are only visible when examined with the electron microscope, rather than the optical microscope.

Hull & Goldsmith (2008) noted that the secondary causes have histologic patterns that are similar to that of the primary causes, with a few variations, such as the presence of inclusion bodies, and their descriptions are typically contingent on their underlying causes – drugs, genetic disorders, cancer, vasculitis, multiple myeloma, amyloidosis, human immunodeficiency virus (HIV), Sjorgen's syndrome, hepatitis B, syphilis, sarcoidosis, systemic lupus erythematosus (SLE), and diabetic nephropathy.

As respects drugs, individuals with kidney failure have been noted to have their proteinuria exacerbated when given captopril or penicillin, or even when there is an accumulation of metals. In terms of genetic disorders, alterations occur in the protein nephrin, which is part of the glomerular filtration barrier, have been observed in congenital forms of the syndrome. Similarly, cancerous cells interfere with the normal function of the glomeruli when they invade them. Vasculitis in the glomeruli also serves as an impediment to the flow of blood in the kidneys, causing kidney damage. Besides, amyloidoisis modifies glomerular shape, and as a consequence, their function. During HIV infection, glomerular function is altered by action of viral antigens that obstruct the lumens of the capillaries of the glomeruli; a similar occurrence is seen during hepatitis B infection. In Sjogren's syndrome, as well as in the case of SLE, there is glomerular inflammation owing to deposits of immune compelxes; a similar occurrence is occasionally seen during sarcoidosis. As regards diabetic nephropathy, inflammation of the

kidney occurs, resulting from the accumulation of excess blood sugar, interfering with normal kidney function.

2.3 Juxtaposing paediatric and adult-onset nephrotic syndrome

There are several points of divergence between paediatric and adult-onset nephrotic syndrome, and these have shaped recommendations regarding how the disease should be managed in these two age-categorized patient groups. For instance, nephrotic children who are between the ages of one and sixteen years are 95% more likely to respond to a sixteen-week glucocorticoid treatment regimen (International Study of Kidney Disease in Children, 1981). Hence in nephrotic children, rather than conduct routine kidney biopsy immediately, the procedure is reserved for cases of relapse or steroid-resistant nephrotic syndrome, particularly, as most paediatric nephrotic cases are cases of minimal change disease, and should paediatric cases of focal segmental glomerulosclerosis occur, they usually represent cases of treatment resistance (Bonilla-Felix *et al*., 1999; Filler *et al*., 2003). In adults, the syndrome is much more heterogenous, involving diversified combinations of demographic features and any of the secondary and primary forms of the syndrome, accounting for why in this patient group, routine kidney biopsy forms part of the early management strategies, as it is a critical step that allows for a more accurate disease categorization and treatment (Korbet *et al*., 1996; Haas *et al*., 1997).

There is an additional disparity between these two patient groups with regard to genetic causes of nephrotic syndrome. With the passing years, monogenetic defects, predominantly those that involve genes essential to the function of podocytes, produce focal segmental glomerulosclerosis on histological examination, and transmitted via autosomal recessive

means, seem to have increased the case counts of paediatric nephrotic syndrome, including neonatal cases (Benoit *et al*., 2010; Trautman *et al*., 2015). Consequently, genetic testing has been suggested as a means of predicting steroid-sensitive cases, sparing those with steroid-resistant nephrotic syndrome from the potential toxicity of empiric therapy, as well as predicting freedom from post-transplantation relapse (Benoit *et al*., 2010; Gbadegesin *et al*., 2015; Giglio *et al*., 2015). In fact, steroid-resistant nephrotic syndrome arising from monogenetic mutations are being reported at a higher frequency in adults than previously, with greater than ten percent of these occurring where the onset of the disease ranged from thirteen to eighteen years of age (Sadowski *et al*., 2015). Another study conducted among patients in Spain reported a high proportion of primary nephrotic syndrome to have involved mutations, with the median age of onset of the disease being 33 years (Santin *et al*., 2011).

2.4 Minimal change disease in adults

Although the presentation of minimal change disease in adults is similar to its presentation in children, it is characterized by a lower odds for relapse occurrence, usually results in acute kidney damage, and seems to have prolonged remission (Nolasco *et al*., 1986; Mak *et al*., 1996; Tse *et al*., 2003). In a study conducted in China involving a population of 340 minimal change disease patients, the proportion of patients who were steroid-resistant was 9.7%, remission occurred in the rest of the patients within a median of ten weeks, 42% initially relapsed, and those who relapsed frequently or became dependent on the steroids comprised 27% (Szeto *et al*., 2015). A higher morbidity has been reported in certain groups. As an example, in the study of (Waldman *et al*., 2007), a quarter of the 95 adult patients who were attended to at a referral

centre (most of whom had been given steroid treatment) experienced acute kidney injury. In the said patient group, 73% initially relapsed, those who relapsed frequently comprised 41%, and the mean remission time was thirteen weeks.

In three randomized controlled trials, extended steroid therapy among nephrotic children was demonstrated to only delay the recognition of frequent relapse and steroid-dependence, but could not prevent it (Teeninga *et al*., 2013; Sinha *et al*., 2015; Yoshikawa *et al*., 2015). It is noted that there is limited data on direct comparison of steroids vis-a-vis alternative agents regarding efficacy (Nolasco *et al*., 1986; Mak *et al*., 1996; Waldman *et al*., 2007). Moreover, steroid intolerance and contraindications regarding steroids, such as osteoporosis and diabetes, are frequently reported in adults (Radhakrishnan & Cattran, 2012). Also, it has been demonstrated that cyclophosphamide treatment increased the risk of cancer development by more than three folds (van den Brand *et al*., 2014).

2.5 Focal segmental glomerulosclerosis in adults

In adult focal segmental glomerulosclerosis, even though proteinuria may occur at levels that qualify it to be associated with nephrotic syndrome, there is usually absence of edema; this is rare in idiopathic focal segmental glomerulosclerosis, but is a common occurrence in the secondary variant (Praga *et al*., 1999). Given that in such cases, the prognosis is excellent, having more than a 90% rate of kidney survival, the therapeutic regimen is more conservative rather than involving immunosuppression (Cameron *et al*., 1978; Korbet *et al*., 1994; Rydel *et al*., 1995). Treatment of the steroid-resistant variant of the condition howbeit poses a challenge, as tacrolimus use is not recommended because there is limited data to support its administration

as a substitute to cyclosporine. In a six-month study comparing monthly administration of tacrolimus and cyclophasphamide intravenously among steroid-receiving adults with focal segmental glomerulosclerosis, the two study groups had improvements in their serum albumin and proteinuria, along with stable GFR, without differing significantly from each other (Ren *et al*., 2013). In another suchlike study that was conducted among steroid-resistant nephrotic children, tacrolimus demonstrated significantly fewer serious infections, shorter remission time, and higher remission rates (both for partial and complete remission) (Gulati *et al*., 2012). Partial and complete remission were also recorded for 52.3% of steroid-resistant focal segmental glomerulosclerosis adult patients receiving 24 weeks of tacrolimus treatment (Ramachandran *et al*., 2014). It is noted though that that study was uncontrolled.

2.6 A highlight of complications of nephrotic syndrome

Several complications have been identified in connection with nephrotic syndrome. For instance, owing to a loss of the binding protein for vitamin D, there is a possibility of occurrence of vitamin D deficiency (Xiang *et al*., 2005; Adams *et al*., 2008). This deficiency has a consequence on calcium levels in the blood, as vitamin D is a regulator of the calcium levels – a reduction in vitamin D levels translates to a reduction in levels of calcium (hypocalcaemia), which although could potentially result in tetany, has been noted to be partly influenced by the levels of albumin (Usatii *et al*., 2007). Another known complication is hypothyroidism, which has been attributed to a reduction in thyroid binding globulin (Sudha *et al*., 2013). Pulmonary edema has also been identified as a complication, and has been noted to be due to a reduction in oncotic pressure following plasma protein loss, and is characterized by dyspnoea and hypoxia

(Bisno *et al*., 1995). Other known complications include Cushing's syndrome, growth retardation, bone abnormalities, hypercoagulability mycrocytic hpochromic anaemia, infections, thromboembolism, atherosclerosis (Basma *et al*., 1999; Kodner, 2009; Satinder *et al*., 2017; Semwal, 2020). Acute kidney failure could also occur, and may be sepsis- or hypovolemia-caused or result from an underlying glomerulonephritis (Goyal *et al*., 2020). Edema of the kidneys that causes a pressure-mediated decrease in the estimated glomerular filtration rate (GFR) also contributes to the fluid storage and weak kidney function (Goyal *et al*., 2020). Further repercussions encompass fluid retention-associated hypertension and kidney function reduction, and gut edema, from which may arise absorption defects, with malnutrition as a consequence, as well as the development of pleural effusions and ascites (Semwal, 2020).

2.7 Specific complications associated with nephrotic syndrome

2.7.1 Hyperlipidemia and atherosclerosis

A typical feature of the syndrome is hyperlipidemia, and is associated with the characteristic hyperproteinemia and reduced oncotic pressure, a consequence of which is reactive synthesis of lipoproteins and other kidney proteins (Appel *et al*., 1985). Additionally, the plasma concentration of lipoprotein lipase is reduced, culminating in lipid catabolism reduction. There is glomerular filtration of a proportion of the increased lipoproteins in the serum, resulting in lipiduria and presence of fatty casts and oval fat bodies in the urine sediment (Sinnakirouchenan, 2020). The syndrome predisposes to atherosclerotic vascular disease – intensive studies indicate the rate and extent of coronary artery stenoses to be higher within this patient group (Curry Jr & Roberts, 1977).

2.7.2 Hypocalcemia

Hypocalcemia frequently occurs in nephrotic syndrome, driven by hypoalbuminemia. Notwithstanding, abnormal bone histology and density also occur in the syndrome, probably due to leakage of proteins responsible for vitamin D binding into urine, resulting in low vitamin D levels and decreased absorption of calcium from the intestines (Mittal *et al*., 1999).

Tessitore *et al*. (1984) have previously noted that even with normal GFR, nephrotic patients have inconsistent calcium levels and abnormalitites in their bones. This was subsequently corroborated in a study involving nephrotic adults (Mittal *et al*., 1999).

2.7.3 Hypercoagulability

Pulmonary embolism and venous thrombosis are both known to be complications of the syndrome. The observed hypercoagulability is resultant of a concurrence of the leakage of plasminogen, antithrombin II, and other anticoagulants into urine and a rise in the levels of factors X, VIII, VII, I, and other clotting factors (Kauffmann *et al*., 1978; Rabelink *et al*., 1994). According to one study conducted by Bellomo & Atkins (1993), about two-fifths of membranous nephropathy patients experience venous thromboembolism. Another study reported renal vein thrombosis and deep vein thrombosis to respectively develop in 25%–30% and 15% of individuals suffering from nephrotic syndrome, and the risk increases across those with minimal change disease (24%), membranoproliferative glomerulonephritis (26%), and membranous glomerulonephritis (37%).

In one cohort of 298 individuals suffering from nephrotic syndrome, who were retrospectively studied by Mahmoodi *et al*. (2008), the researchers reported arterial and venous

thromboembolism to have occurred at an annual incidence of 1.5% and 1% respectively, a significant increase over the 1–2 per 1000 persons annualy known to exist in the general population (Scheres *et al*., 2018), similar to what was contained in a contemporary report (Kayali *et al*., 2008). As opposed to the venous thrombosis risk, for which proteinuria was implicated, the arterial risk was linked to reduced GFR, smoking, diabetes, hypertension, and other arterial disease determinants. These risks were reportedly higher within the initial six months following diagnosis. Even so, there are some variations in these risks, mediated by patient-specific factors, with an associated thirty days death incidence of 6%–12% after pulmonary embolism and deep vein thrombosis (White, 2003; Ageno *et al*., 2006).

2.7.4 Hypovolemia

Hypovolemia is resultant of oncotic pressure reduction-induced hypoalbuminemia, which as a consequence, causes water loss from plasma into the interstitium, with a reduction in circulating blood volume. Typically, it is observed in cases of hypoalbuminemia that falls below 1.5 g/dL. Its symptoms encompass cold hands and feet, diarrhea, abdominal pain, vomitting, tachycardia, oliguria, and delayed capillary filling, with a later occurrence of hypotension (Satinder *et al*., 2017; Semwal, 2020).

2.8 Alpha-2-macroglobulins

Alpha-2-macroglobulin (A2M) is synthesized in the liver, and is a large tetrameric 35-amino acid ‘bait’ region-containing plasma protein, whose structure is maintained by disulphide bonds, and wit. It is capable of inactivating a diverse range of proteinases, such as aspartic-,

cysteine-, serine-, and metalloproteinase (Rehman *et al*., 2013). The 'bait' region is bound and cleaved by proteinases to form A2M-proteinase complexes, which are recognized by receptors on macrophages to facilitate their clearance. The active forms of gelatinase – MMP-2 and MMP-9 – are also bound to and cleared by A2M with the aid of phagocytes' scavenger receptors. Owing to the larger size of A2M, it is retained in circulation during the leakage of proteins in nephrotic syndrome (Topal *et al*., 2014). Its levels also rise during the synthesis of all proteins and these high levels are seen during pregnancy, as well as cirrhosis and diabetes (Topal *et al*., 2014).

2.9 Oncotic pressure of blood plasma

Oncotic pressure is the osmotic pressure achieved from the difference within the extracellular fluid between the protein contents of the plasma and the interstitial fluid (Lent-Schochet & Jialal, 2020). Oncotic pressure is the osmotic pressure generated by large molecules (especially proteins) in solution. It is indicated that the correlation to van't Hoff's law is more precise with small, globular proteins than with larger protein molecules. Some 90% by weight of plasma is water and about 8% is plasma proteins (albumin, globulins, and fibrinogens).

Blood plasma is an aqueous solution containing different ions (Na^+, K^+, Ca^{2+}), small non-dissociated molecules (glucose, amino acids) and proteins macromolecules (albumin, globulin etc).

Each type of molecule contributes a specific osmotic pressure, the sum representing the colloid-osmotic pressure or oncotic pressure of plasma.

$$\pi plasma = \sum \pi mol + \sum \pi ions + \sum \pi proteins$$

Because of the lower molecular weight and higher plasma concentration of albumin, it is the predominant determinant of oncotic pressure (Guyton & Hall, 2006).

2.10 Formation of tissue fluid

Filtration occurs at the arterial ends of capillaries, as oncotic pressure of plasma proteins are overcome by blood hydrostatic pressure; conversely, as reabsorption occurs at the venous ends, at which sites the oncotic pressure of plasma proteins are higher than the blood hydrostatic pressure – formation of tissue fuild is the net outcome of these filtrations (Lent-Schochet & Jialal, 2020). Albumin contributes to about 80% of the oncotic pressure of the plasma, and it possesses greater anionic charge (Holbeck *et al*., 2001). The phenomenon described above only occurs as such only when the fluid in the interstitial space lacks high levels of proteins – should there be a higher concentration of protein there (or conversely if loss of proteins occur from the intravascular space), there is a fall in oncotic pressure, resulting in easier migration of fluid between the interstitial and intravascular spaces, causing edema (Lent-Schochet & Jialal, 2020).

2.11 Generation and maintenance of colloid osmotic pressure

Although other proteins, such as fibrinogen and immunoglobulins, determine the oncotic pressure, the key contribution comes from albumin, contributing to approximately 80% of plasma COP (Holbeck *et al*., 2001). These plasma proteins are concentrated within the vasculature, as the capillary membranes are marginally permeable to them, this generates a

significant proportion of the osmotic pressure, arising from the creation of the concentration gradient across the membrane.

The Gibbs-Donnan effect also contributes to COP. Albumins and most other proteins are negatively charged, and surrounded by non-covalently bound cations, such as sodium (Lent-Schochet & Jialal, 2020). These sodium ions act independently from their own concentration gradients and further elevate the COP water-retaining effect within the vasculature, which by virtue of its being additive, rises disproportionately with every rise in the concentration of albumin (Chan *et al*., 2001). Acidemia, which frequently occurs among critically ill patients, reduces the relative negative charge of albumin, restricting the Gibbs-Donnan effect and decreasing colloid osmotic pressure.

Albumin synthesis takes place exclusively in hepatocytes. In situations of adequate nutritional status and ample supply of amino acids, albumin synthesis is thought to be regulated by hepatic plasma COP. However, other factors independent of COP may also be involved in albumin synthesis (Schmid *et al*., 1986).

Edema results when there is excess accumulation of tissue fluids, either within the collagen-mucopolysaccharide matrix distributed in the interstitial spaces (interstitial edema) or within cells (cellular edema) or (Lent-Schochet & Jialal, 2020).

CHAPTER THREE

3.0 METHODOLOGY

3.1 Study design

A case-control study design was used for the study.

3.2 Study site

The study was carried out at MDS-Lancet Laboraroties Ghana Limited. The facility is ISO Standard 15189:2012-certified, has over eighteen branches spread across Ghana, and provides specialist medical laboratory services for hospitals, clinicians, corporate groups, non-governmental organizations (NGOs), insurances, educational institutions, research teams, and occupational health establishments, and has several departments, including pre- and post-analytical department, chemical pathology, haematology, microbiology, molecular biology, and histopathology. On the average, the facility attends to between 800 and 1,500 patients daily.

3.3 Study population and recruitment

In this study, ninety-nine (99) individuals with nephrotic syndrome and 47 individuals without the condition (serving as the control group) were recruited at MDS-Lancet Laboraroties Ghana Limited. Individuals who had been diagnosed with nephrotic syndrome by their clinicians, of whom further laboratory tests had been requested to assist with their care were sampled as part of the nephrotic syndrome group. Those recruited as part of the control group were apparently healthy individuals who were undergoing general medical check up at the facility. The sociodemographic and clinical data of the study participants were gathered by means of a standard questionnaire (Appendix II) and a review of patients' laboratory request forms.

3.4 Inclusion criteria

All participants with clinical confirmation of the presence of nephrotic syndrome were recruited as part of the nephrotic syndrome group. The control group participants were screened and were expected to lack the clinical features and characteristics of nephrotic syndrome. All the study participants were required to give their consent for inclusion in the study.

3.5 Exclusion criteria

Individuals with diabetic and renal complications, immunocompromised individuals, those with presence of severe disease (any condition requiring immediate medical treatment as determined by medical personnel or researchers), and individuals who were mentally unstable were excluded from the study.

3.6 Sample size determination

The determination of the minimum sample size was done using the formula:

$$n = \frac{z^2 \times p\,(1-p)}{m^2}$$

Where n = Minimum sample size

z = Confidence level at 95% (standard value of 1.96)

m = Margin of error at 5% (standard value of 0.05)

p = Estimated prevalence of nephrotic syndrome = 5.0%.

Hence $$n = \frac{1.96^2 \times 0.05\,(1-0.05)}{m^2} = 72.99 \approx 73$$

3.7 Clinical and anthropometric assessment

Socio-demographic and clinical data of the study participants were gathered by means of a standard questionnaire (Appendix II) and a review of patients' folders. For all participants, body weight and height were measured using a standard physician's scale and a wall-mounted meter rule, to the nearest 1.0 kilograms (kg) and 0.005 metres (m) respectively. The body mass indices (BMIs) of the participants were calculated as weight/height (kg/m^2).

3.8 Laboratory sampling and processing

Six milliliters (6 mls) of venous blood sample was taken from each participant by a phlebotomist, following acceptable medical techniques to avoid hemolysis. Two milliliters of each blood sample was put into an ethylene diamine tetra acetic (EDTA)-containing tube, and the remaining 4mls was put into a gel separator tube. Samples were taken between 7:00 am and 9:00 am each day. All the samples were then transported to the working area of the MDS-Lancet laboratories Ghana Limited head office for processing.

The samples in the gel separator tubes were allowed to clot, and subsequently centrifuged at 3000 rpm for 10 minutes at room temperature to allow the sera to separate. Each serum sample was aliquoted into 0.5ml portions and stored at -20 °C up to six months, and were used to determine concentrations of albumin and non-albumin protein fractions. Electrophoresis technique was also used to separate the various proteins, and the various fractions determined using the densitometer.

A semi-automated multi-parameter system was used for start-to finish. The agarose gel electrophoresis involved application of samples, migration, incubation, drying, staining, destaining and final-stage drying. The software allows for control of the instrument (migration, staining) with a touch screen. The instrument is also connected to a computer with the "PHORESIS" software, which allows the operator to display the image of the electrophoretic migration. This has been illustrated in Figure 1 below, for a known pathological patient.

The oncotic pressure calculation was done using the formula $COPpl = \alpha (2.8c + 0.18c2 + 0.012c3) + \beta (0.9c + 0.12c2 + 0.004c3)$, where α and β respectively represent albumin and

globulin fraction, and "c" represents concentration in g/l. The last (generalized for plasma with any protein compositions) closely corresponded to the values directly measured in normal human plasma with variable protein compositions. This was developed on a nomogram for practical use (the corrected protein equation to estimate plasma colloid osmotic pressure and its development on a nomogram).

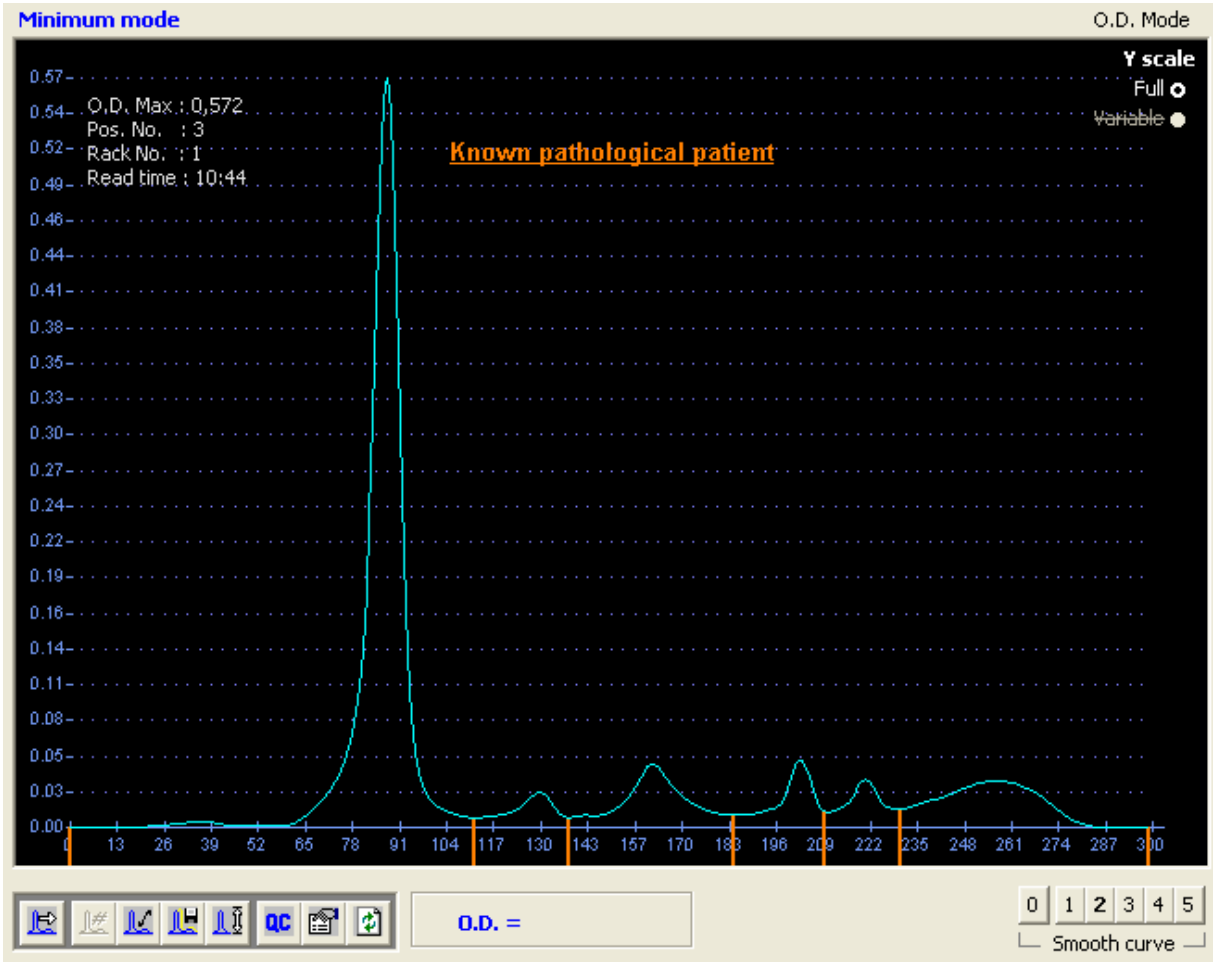

Figure 3.1: An illustration of the electrophoresis technique used in this study

3.9 Principles of tests

3.9.1 Urea

Urea is hydrolysed in the presence of water and urease to produce ammonia and carbon dioxide (Chaney *et al*., 1962; Searcy *et al*., 1967). The ammonia produced in the first reaction combines with 2-oxoglutarate and NADH in the presence of glutamate-dehydrogenase (GLDH) to yield glutamate and NAD^+. The decrease in NADH absorbance per unit time is proportional to the urea concentration.

A summary of the reaction steps is presented below:

$$\text{Urea} + H_20 \xrightarrow{\text{Urease}} 2NH_3 + CO_2$$

$$NH_3 + 2 - \text{oxoglutarate} + \text{NADH} \xrightarrow{\text{GLDH}} \text{Glutarate} + NAD^+$$

3.9.2 Creatinine

Creatinine forms a yellow-orange coloured compound with picric acid in an alkaline medium. The rate of change in absorbance at 520/800 nm is proportional to the creatinine concentration in the sample.

A summary of the reaction steps is presented below:

$$\text{Creatinine} + H_20 \xrightarrow{\text{Creatinine amidinohydrolase}} \text{Creatine}$$

$$\text{Creatine} + H_20 \xrightarrow{\text{Creatinine amidinohydrolase}} \text{Sarcinose} + \text{urea}$$

$$\text{Sarcosine} + O_2 + H_20 \xrightarrow{\text{Sarcosine oxidase}} \text{Glycine} + \text{formaldehyde} + H_2O_2$$

$$H_2O_2 + \text{Leuco dye} \xrightarrow{\text{Peroxidase}} \text{Dye} + 2H_20$$

3.9.3 Albumin

A coloured complex is formed when bromocresol green reacts with albumin. The absorbance of the albumin-BCG complex is measured bichromatically (600/800nm) and is proportional to the albumin concentration in the sample. A summary of the reaction is presented below:

$$\text{BCG} + \text{Albumin} \xrightarrow{\text{pH 4.3}} \text{BCG} - \text{albumin complex}$$

3.9.4 C-reactive proteins

During inflammation, the immune cells, particularly, those of the innate immunity produce cytokines including IL-1, IL-6, and TNF-α, which subsequently activate the hepatocytes to produce CRP and other acute phase proteins (Mahmoud & Rivera, 2002; Guo *et al*., 2015). Using the particle enhanced turbidimetric assay, which is sensitive enough to detect even low levels of CRP, human CRP agglutinates with latex particles coated with monoclonal anti-CRP antibodies. The precipitate is determined turbidimetrically at 552 nm.

3.9.5 Transferrin

Human transferrin forms a precipitate with a specific antiserum which is determined turbidimetrically at 340 nm.

3.9.6 Calcium

The basis of this method is the specific binding of the metallochromic indicator, cresolftalein complex one (OCC), and calcium at alkaline pH with the resulting shift in the absorption wavelength of the complex. The intensity of the cromophore formed is proportional to the concentration of total calcium in the sample.

$$\text{OGC} + \text{Calcium} \rightarrow \text{OGC} - \text{calcium complex}$$

The reagents and samples were brought to room temperature, following which they were pipetted into labeled test tubes and mixed. The tubes were then allowed to stand at room temperature for two minutes, and their absorbance read at 570 nm wavelength.

3.10 Data processing

The data obtained in this study was entered into Microsoft Excel and exported to the Statistical Products and Services Solutions (SPSS), version 25 for analysis. The sociodemographic and clinical data obtained, as well as data on the serum levels of the albumin and non-albumin proteins were summarized using descriptive statistics. Independent-samples t-test was used to determine the differences in the levels of albumin, non-albumin proteins, and oncotic pressure between the study groups, at an alpha level of 0.05. The Eta squared statistic was also computed

to determine the effect size of the observed differences, and interpreted following Cohen's (1988) guidelines (0.01 = small effect; 0.06 = moderate effect; 0.14 = large effect). Finally, a binary logistic regression analysis was conducted to determine predictors of occurrence of nephrotic syndrome among the study participants. The significance of each predictor was evaluated by determination of the *p* values, odds ratio, and confidence intervals; *p* values whose magnitude fell below 0.05 were considered statistically significant.

3.11 Ethical considerations

Ethical approval for this study **(ID: CHS-Et/M2-5.5/2019-2020)** was obtained from the Ethical and Protocol Review Committee (EPRC), College of Health Sciences (CHS), University of Ghana. Informed consent (Appendix I) was also obtained from all potential study participants before their enrolment into the study. Risks and benefits associated with enrolment in the study were explained adequately to the study participants, and the information collected from them were stored on a password-protected computer, to ensure confidentiality.

CHAPTER FOUR

4.0 RESULTS

4.1 Sociodemographic and clinical features of the study participants

In this study, 146 individuals volunteered as study participants. These comprised 99 individuals diagnosed with nephrotic syndrome and 47 apparently healthy individuals serving as the control group. The distribution of males was 51.5% (n = 51) for the nephrotic syndrome group and 44.7% (n = 21) making up the control group. The corresponding distribution for females was 48.5% (n = 48) for the nephrotic syndrome group and 55.3% (n = 26) making up the control group. The mean age of the study participants was 46.95±22.19 years in the nephrotic syndrome group and 45.72±16.08 years in the control group, and the corresponding values for body mass index (BMI) were 23.63±3.06 Kg/m^2 and 25.14±3.08 Kg/m^2 respectively. In addition, majority of the study participants were married [64.6% (n = 64) in the nephrotic syndrome group and 85.1% (n = 40) in the control group], had had tertiary education [62.6% (n = 62) in the nephrotic syndrome group and 66.0% (n = 31) in the control group] and had no family history of nephrotic syndrome [84.8% (n = 84) in the nephrotic syndrome group and 91.5% (n = 43) in the control group]. Details of the study participants are presented in Tables 4.1 and 4.2 below.

Table 4.1: Sociodemographic characteristics of the study participants

Features	Nephrotic Group		Control Group		Chi square	*p* value
	Number	%	Number	%		
Gender					0.60	0.44
Male	51	51.5	21	44.7		
Female	48	48.5	26	55.3		
Marital status					6.59	0.04
Single	28	28.3	6	12.8		
Married	64	64.6	40	85.1		
Not applicable	7	7.1	1	2.1		
Educational level					10.75	0.01
None	8	8.1	1	2.1		
Basic	12	12.1	0	0.0		
Secondary	17	17.2	15	31.9		
Tertiary	62	62.6	31	66.0		
Occupation					7.29	0.40
Finance sector	12	12.1	4	8.5		
Trader	23	23.2	16	34.0		
Education sector	12	12.1	10	21.3		
Health sector	0	0.0	3	6.4		
Journalist	0	0.0	1	2.1		
Retired	28	28.3	9	19.1		
Student	10	10.1	3	6.4		
Not applicable	8	8.1	1	2.1		

Table 4.1: Socio-demographic characteristics of the study participants. Data presented in frequency (No.) and percentages (%), p-value < 0.05 was considered significant.

Table 4.2: Clinical features of the study participants

Features	Nephrotic Group		Control Group		Chi square	*p* value
	Number	%	Number	%		
Age					17.65	0.04
0–10	8	8.08	1	2.13		
>10–20	5	5.05	2	4.26		
>20–30	11	11.11	2	4.26		
>30–40	9	9.09	15	31.91		
>40–50	23	23.23	8	17.02		
>50–60	17	17.17	10	21.28		
>60–70	10	10.10	6	12.77		
>70–80	12	12.12	3	6.38		
>80–90	1	1.01	0	0.00		
>90–100	1	1.01	0	0.00		
Family history of nephrotic syndrome					1.24	0.27
Yes	15	15.2	4	8.5		
No	84	84.8	43	91.5		

Table 4.2: Clinical features of the study participants. Data presented as frequency and percentages; BMI = Body mass index; p-value < 0.05 was considered significant; Age [Nephrotic Group $(\overline{X} \pm SD)$ *= 46.95 ± 22.19 years; Control Group* $(\overline{X} \pm SD)$ *= 45.72 ± 16.08 years]; BMI [Nephrotic Group* $(\overline{X} \pm SD)$ *= 23.63 ± 3.06 Kg/m^2; Control Group* $(\overline{X} \pm SD)$ *= 25.14 ± 3.08 Kg/m^2]*

4.2 A comparison of the serum levels of the various albumin and non-albumin proteins

The independent-samples t-test conducted revealed that the serum levels of alpha-2-globulin was marginally significantly higher among the participants of the nephrotic syndrome group than among those of the control group; those of C-reactive protein, urea, gamma globulins, and

calcium were also significantly higher among the participants of the nephrotic syndrome group. A decrease was observed for transferrin, total proteins, albumins, and beta-1-globulins. With regard to the serum levels of creatinine, alpha-1-globulin, alpha-2-globulin, beta-2-globulins, and M component, however, the two groups did not differ significantly. A summary of the results of the independent-samples t-test is presented in Table 4.3 below.

Moreover, the independent-samples t-test conducted revealed that the oncotic pressure of the participants of the nephrotic syndrome group (M = 20.02; SD = 7.41) was significantly lower than that observed for the control group (M = 27.73; SD = 5.40; t (120.02) = -7.12 ; $p < 0.0001$; 95% *CI* = -9.86 – -5.57). The Eta squared statistic computed (Eta squared = 0.30) indicated a large effect size for the differences observed.

Table 4.3: A comparison of biochemical parameters of the study participants

Features	Nephrotic Group		Control Group		*p value*	95% *CI*
	Mean	S.E.M.	Mean	S.E.M.		
C-reactive protein (mg/L)	57.65*	8.34	1.88*	0.44	<0.001	39.20 – 72.33
Creatinine (µmol/L)	198.96	33.95	125.76	28.00	0.10	-13.80 – 160.20
Urea (µmol/L)	9.34*	1.13	5.95*	0.78	0.02	0.67 – 6.12
Transferrin (µmol/L)	20.38*	0.98	33.98*	1.23	<0.001	-16.72 – -10.47
Total proteins (g/L)	61.14*	1.76	68.89*	1.39	<0.001	-13.18 – -4.32
Albumin (g/L)	26.51*	1.11	39.48*	1.18	<0.001	-16.19 – -9.78
Alpha-1-globulin (g/L)	2.27	0.09	1.89	0.35	0.17	-0.17 – 0.92
Alpha-2-globulin (g/L)	7.28*	0.30	6.56*	0.21	0.05	-0.002 – 1.45
Beta-1-globulin (g/L)	4.83*	0.13	5.32*	0.12	0.01	-0.85 – -0.14
Beta-2-globulin (g/L)	3.09	0.14	3.06	0.12	0.89	-0.33 – -0.39
Gamma globulin (g/L)	13.14*	0.86	9.51	0.18*	<0.001	1.90 – 5.36
M component (g/L)	2.41	1.24	0.14	0.14	0.07	-0.21 – 4.75
Calcium	2.08*	0.03	2.41	0.03*	<0.001	-0.42 – -0.24
Oncotic pressure (mmHg)	20.02*	0.74	27.73	0.79	<0.001	-9.86 – -5.57

*Table 4.3: Biochemical features of the study participants. Data presented as mean and SEM to highlight precision of the mean; SEM= standard error of mean; *Significant at 0.05 alpha level*

4.3 Predictors of nephrotic syndrome occurrence among the study participants

The binary logistic regression analysis conducted indicated that among the sociodemographic and clinical features of the study participants, only BMI and serum levels of C-reactive protein, urea, alpha-1-globulin, and gamma globulin significantly predicted the occurrence of nephrotic syndrome. Serum levels of C-reactive protein (OR = 1.41, p = 0.005) and gamma globulin (OR = 4.12, p = 0.005) increased the odds of occurrence of nephrotic syndrome by about one and a half and four folds respectively, whereas BMI (OR = 0.62, p = 0.010) and serum levels of urea (OR = 0.74, p = 0.004) and alpha-1-globulin (OR = 0.40, p = 0.006) were each protective of nephrotic syndrome occurrence – increased levels of each of these biomarkers reduced the

likelihood of nephrotic syndrome occurrence. In Table 4.4 below, the results of the logistic regression analysis are presented.

Table 4.4: Risk factors for occurrence of nephrotic syndrome

Predictors	B	S.E.	Wald	df	*p* value	OR	95% *CI*
CRP	0.34	0.12	7.78	1	0.005	1.41*	1.107 – 1.792
Creatinine	0.000	0.002	0.014	1	0.90	1.000	0.996 – 0.004
Urea	-0.30*	0.10	8.27	1	0.004	0.743*	0.606 – 0.910
Transferrin	-0.143	0.09	2.33	1	0.13	0.87	0.722 – 1.042
Total proteins	-0.367	0.44	0.69	1	0.41	0.69	0.292 – 1.642
Albumin	0.052	0.123	0.176	1	0.674	1.05	0.827 – 1.341
Alpha-1-globulin	-0.92*	0.336	7.56	1	0.006	0.40*	0.206 – 0.767
Alpha-2-globulin	0.188	0.377	0.249	1	0.62	1.21	0.577 – 2.525
Beta-1-globulin	0.614	0.588	1.092	1	0.30	1.85	0.584 – 5.847
Beta-2-globulin	-0.752	0.579	1.682	1	0.20	0.472	0.151 – 1.469
Gamma globulin	1.415	0.506	7.833	1	0.005	4.117*	1.528 – 11.091
M component	0.343	0.359	0.914	1	0.339	1.409	0.698 – 2.846
Family history of nephrotic syndrome	1.537	1.030	2.229	1	0.135	4.652	0.618 – 35.00
Alcohol consumption	0.105	0.786	0.18	1	0.893	1.11	0.238 – 5.184
Gender	0.446	0.862	0.268	1	0.605	1.563	0.288 – 8.464
Oncotic pressure	0.290	0.730	0.158	1	0.691	1.337	0.320 – 5.594
BMI	-0.485	0.189	6.589	1	0.010	0.615*	0.425 – 0.892
Calcium	-3.127	1.856	2.838	1	0.092	0.044	0.001 – 1.667

*Table 4.4 shows the predictors of nephrotic syndrome of study participants.; *Significant at 0.05 alpha level; S.E. = Standard error; df = 1; OR = Odds ratio; CRP = C reactive protein; BMI = Body mass index*

CHAPTER FIVE

5.0 DISCUSSION

A key objective of this study was to compare individuals diagnosed with nephrotic syndrome to apparently healthy individuals with regard to their levels of albumin and non-albumin proteins, including C-reactive protein, urea, transferrin, total proteins, creatinine, alpha-1-globulin, alpha-2-globulin, beta-1-globulins, beta-2-globulins, gamma globulins, and M component. As was observed in this study, the serum levels of C-reactive protein, urea, gamma globulins, alpha-2-globulins, and calcium were significantly higher in the nephrotic syndrome group compared to the control group, whereas the levels of transferrin, total proteins, albumins, and beta-1-globulins were significantly lower in the case group.

This co-occurrence of high C-reactive protein levels and low albumin levels among the participants of the nephrotic syndrome group is in line with previous studies. To illustrate, in patients undergoing dialysis, an association between high C-reactive protein levels and lower serum albumin levels have been reported (Kaysen *et al*., 1995; Kaysen *et al*., 1997; Yeun & Kaysen, 1997).

It is also noted that low serum albumin levels is a key culprit of hypocalcemia (which is an hallmark of nephrotic syndrome), and hence a strong case could be made that this cohort of nephrotic patients could be at risk of hypocalcemia (Mittal *et al*., 1999). The observance of a higher serum concentration of calcium in the nephrotic patients, but not similar levels to the control group indicates that improvements need to be made with regard to their management.

Reduced levels of albumin among nephrotic patients, particularly, levels below 1.5 g/dL, is further implicated in hypovolemia; in the current study, the albumin levels of the nephrotic patients, although being lower than that observed in the control group, seem not to necessarily predispose them to hypovolemia – the mean albumin level was 26.51 g/dL; plus, the recorded standard deviation for the biomarker was 11.09, suggesting that the participant with the lowest level of albumin among the nephrotic patients recorded albumin levels of 4.41 g/dL, which falls above the 1.5 g/dL threshold noted above.

Another important objective of the current study was to determine whether the individuals diagnosed with nephrotic syndrome and the apparently healthy individuals significantly differed from each other as respects their colloid osmotic pressures. It was shown that the oncotic pressure of the nephrotic syndrome group was significantly lower than that observed for the control group. This was expected, as the nephrotic syndrome-diagnosed individuals were confirmed to have significantly lower levels of albumin. It has been reported in previous studies that albumins are principally involved in maintaining colloid oncotic pressure. Holbeck *et al*. (2001), for instance, reported that it accounts for approximately 80% of the colloid osmotic pressure, and this has been attributed to its being the lowest molecular weight protein among the major plasma proteins, and being of a concentration that is almost double that of globulin. Interestingly, colloid osmotic pressure has been suggested as a regulator of albumin synthesis (Schmid *et al*., 1986). Moreover, C-reactive protein, urea, alpha-2-globulins, and gamma globulins, though significantly higher among the nephrotic syndrome group, seemed not to be able to maintain the colloid oncotic pressure – this apparently serves as another line of evidence obtained from the current study that confirms earlier reports about albumins being the key maintainers of colloid oncotic pressure.

A third objective of the current study was to determine predictors of nephrotic syndrome occurrence among the study participants. In this study, higher levels of C-reactive protein and gamma globulin increased the odds that an individual would be diagnosed with nephrotic syndrome by about one and a half and four folds respectively. Conversely, increasing BMI and serum levels of urea and alpha-1-globulin each reduced the likelihood of nephrotic syndrome occurrence. The huge gaps in knowledge regarding nephrotic syndrome appear to include these observations, as this study seems to be the first to yield such insights about the epidemiology of nephrotic syndrome. It is however noted that the conduction of more suchlike studies could shed more light on these observed phenomena, and could bring to the fore other interesting findings.

CHAPTER SIX

6.0 CONCLUSIONS, RECOMMENDATIONS, AND LIMITATIONS

6.1 Conclusions

The nephrotic syndrome group were found to have lost C-reactive protein, urea, gamma globulins, and calcium into the serum, co-occurring with a lower colloid osmotic pressure and serum levels of transferrin, total proteins, albumins, and beta-1-globulins compared to the control group. Serum levels of the other proteins investigated didnot differ significantly between the two groups.

Serum levels of C-reactive protein (OR = 1.41, p = 0.005) and gamma globulin (OR = 4.12, p = 0.005) increased the odds of occurrence of nephrotic syndrome by about one and a half and four folds respectively.

6.2 Recommendations

Based on the findings in this study, it is recommended that this study be replicated using larger sample sizes, with adjustment in methodology to include investigating urine samples and lipidemia.

6.3 Limitations

This study had a few limitations. First, hyperlipidemia, which is related to serum osmotic pressure of nephrotic syndrome, was not investigated, and hence this study cannot account for

the dynamics that hyperlipidemia introduces to the epidemiology of nephrotic syndrome. Moreover, urine samples were not collected and investigated as part of this study, and hence the study doesnot paint a full picture of the epidemiology of nephrotic syndrome.

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APPENDIX I

RESEARCH CONSENT FORM

Investigator: Mr. Egyam Bill Clinton

Institution: Department of Chemical Pathology, University of Ghana Medical School (UGMS)

Research title: Serum protein determinants and oncotic pressure in Ghanaian patients with nephrotic syndrome

Invitation to participate in the research:

Nephrotic syndrome is a medical condition that affects millions of people worldwide. Among individuals suffering from the condition, proteins that play important roles in the body leak into the urine. One of the proteins that leak out is called albumin, and a lot of research has been carried out on it. However, very little is known about the other proteins that leak into the urine and how they can predict severity of nephrotic syndrome. Such information would help to improve the management of nephrotic syndrome patients. Therefore, this research aims at determining the levels of proteins other albumin in the blood and urine of people with and without nephrotic syndrome.

You are invited to partake in this study. It would involve the collection blood and urine samples from you. Your answers to a few questions would also be needed.

Duration of the study: The samples would be collected within a three-month period and taken to the laboratory for analysis.

Benefits of the study:

This study would provide insights on oncotic pressure as well as serum and urine concentrations of various proteins in relation to albumin among individuals with and without nephrotic

syndrome in Ghana. This information would help in the improvement of the management of nephrotic syndrome patients.

Use of collected material: The urine and blood samples would be analyzed to determine the concentrations of the different proteins under investigation.

Potential hazards of the study:

You may experience some amount of pain during the collection of the blood samples from you. You might also have a little bruising at the sight of blood draw, which will be cared for by qualified health personnel.

Participant's right to refuse or withdraw:

If you have read this form, or the content herein has been clearly explained to you, and you have agreed to participate in this study, please note that participation is voluntary, and you have the right to withdraw your consent or discontinue participation in the project at any time without penalty. It is also your right to refuse to answer questions you are not comfortable with.

Confidentiality:

All collected materials from you will be coded using numbers and letters. Your privacy will be maintained in all published and written data resulting from the study.

Questions, concerns, or complaints:

If you have any issues relating to this study, please direct them to Mr. Egyam Bill Clinton (0243544065), Dr. Seth Amanquah (0244293987), or Dr. Emmanuel Ofori (0244267217), of the Department of Chemical Pathology, UGMS.

Consent for inclusion: If you agree to your inclusion in this study, please complete the form below:

I .....…… on this day …….. (Day/Month/Year) attest that I understand the explanations given in the consent form and thus give permission to Mr. Egyam Bill Clinton to include me in the research study titled "Serum protein determinants and oncotic pressure in Ghanaian patients with nephrotic syndrome".

Signature of participant: …….....

Contact address: .....……..

Phone number: …….....

Thumb print (where required)

APPENDIX II

PARTICIPANTS' QUESTIONNAIRE

PARTICIPANT'S CODE: ..... **DATE: .....**

SECTION 1: SOCIO-DEMOGRAPHIC DATA

1. Gender Male [ ] Female [ ]

2. Age ……...

3. Religion. Christian [ ] Muslim [ ] African Traditionalist [ ] Other .....

4. Traditional Language …….....

5. Marital Status Single [ ] Married [ ] Divorced [ ] Separated [ ] Widowed [ ]

6. Number of Children …….....

SECTION 2: SOCIO-ECONOMIC DATA

7. Employment status Employed [ ] Unemployed [ ] Retired [ ]

8. Occupation .....

9. Educational level Primary [ ] JHS [ ] SHS [ ] TERTIARY [ ]

10. How would you rate the impact of nephrotic syndrome on your personal finances?

Little impact [ ] Moderate impact [ ] Severe impact

SECTION 3: CLINICAL AND RELATED DATA

11. Presence of family history of nephrotic syndrome Yes [ ] No [ ]

12. Do you have any of the following conditions?

Hyphenation [ ] Cardiovascular condition [ ] Diabetes [ ] Other [ ] – please specify……..

13. How often do you visit the hospital? …….

14. Knowledge about nephrotic syndrome

No idea [ ] Knowledgeable [ ] Very knowledgeable [ ]

15. Knowledge about nephrotic syndrome

No idea [ ] Knowledgeable [ ] Very knowledgeable [ ]

16. What medication(s) are you on? …….

17. Since when have you been diagnosed of nephrotic syndrome? …….

18. Do you drink alcohol? Yes [ ] No [ ]

19. Have you had your oncotic pressure measured before? Yes [ ] No [ ]

20. If your answer to (19) above is yes, what was the result? …….