University of Gezira

Performance of Immunofluorescence on Paraffin Wax Embedded Sections in The Diagnosis of Lupus Nephritis Compared to Immunofluorescence on Frozen Sections

(2011-2013)

By

Omer Hassan Omer Ismail

April, 2014
Performance of Immunofluorescence on Paraffin Wax Embedded Sections in The Diagnosis of Lupus Nephritis Compared to Immunofluorescence on Frozen Sections

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Omer Hassan Omer Ismail

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April, 2014
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Date of Examination:  16 / 4 /2014
Declaration

This study was carried out by my own effort at the university of Gezira, Sudan under the supervision of Dr. Ali Seed Ahmed Mohammed. The renal biopsies and data collection has been carried out in the department of renal dialysis, Sudan. The immunofluorescence studies were carried out in the department of histopathology and cytology in Omdurman military hospital, Sudan.
TO My Family

To My Friends

To Every One Who Suffers From Lupus
ACKNOWLEDGEMENT

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Performance of Immunofluorescence on Paraffin Wax Embedded Sections in The Diagnosis of Lupus Nephritis Compared to Immunofluorescence on Frozen Sections

(2011-2013)

Omer Hassan Omer Ismail

Abstract

Systemic lupus erythematosus (SLE), is a chronic inflammatory autoimmune disease with multisystem involvements, it often harms the heart, joints, skin, lung, blood vessels, liver, kidneys and nervous system. The laboratory diagnosis depends on the routine stain (Haematoxylin and Eosin), and ancillary studies such as immunofluorescence, immunohistochemistry and electron microscopy. The disease may occur at any age, but the most common between the age of 15 and 40 years with a female preponderance of 4 to 1. There is no one specific cause of SLE, there are, however, a number of environmental triggers and a number of genetic susceptibilities. The accurate diagnosis of SLE is based on a combination of clinical finding and laboratory evidence. The objective of the present study was to apply the Immunofluorescence technique on paraffin sections to help in diagnosis of SLE in Sudan, because the Immunofluorescence technique on frozen and paraffin sections for immunoglobulins and complement is often the primary approach for the differential diagnosis of glomerular diseases. We carried out the study between March 2011 to April 2013, on 64 patient with lupus nephritis, and the Immunofluorescence technique on frozen and paraffin sections were used to study the renal biopsies. A computer program was used for data analysis. Fifty four patients were females (84.4%) and ten were males (15.6%). This study revealed that the sensitivity of Immunofluorescence on frozen sections (81.3%) was slightly higher than Immunofluorescence on paraffin sections (70.3%). Conclusively, frozen section technique can be effectively replaced by paraffin wax technique, if some modifications are introduced to reach the optimum time in order to achieve a high sensitivity.
تطبيق تقنية التألق المناعي على مقاطع الشمع البارافيني فى تشخيص مرض الذئبة الكلوية ومقارنتها مع تقنية التألق المناعي على المقاطع المجمدة (2011-2013)

عمر حسن عمر إسماعيل

ملخص الدراسة

مرض الذئبة الحمراء المجموعى هو مرض مناعة ذاتية التهابى مزمن يصيب القلب و المفاصل و الجلد والرئتين والاوعية الدموية والكبد والكلى والنهايات و الجهاز العصبي وهو مرض شائع الحدوث بين سن الخامسة عشر والأربعين ونسبة حدوثه في الإناث أعلى من الذكور (نسبة 4:1) لا يوجد سبب محدد لهذا الداء ويعتقد أنه بسبب مجموعة من العوامل البيئية و الوراثية. الهدف الأساسي من هذه الدراسة هو تطبيق تقنية التألق المناعي على العينات المطمورة بالشمع البارافينى المأخوذة من خزعات الكلى. للمساعدة في التشخيص الدقيق لهذا الداء في السودان، ولتقنية التألق المناعي على المقاطع المجمدة والمقاطع المطمورة بالشمع البارافيني للكشف عن القلوبيولينات المناعية والمكونات المكملة الوسيلة الأولى في التشخيص التفريقي لأمراض الكلى. أجريت هذه الدراسة في الفترة ما بين شهر مارس 2011 وحتى شهر ابريل 2013 في قسم أمراض الكلى والرياضيات الدمى بمستشفى الصلح الطبي أدمدان. أجريت الدراسة على 64 مريضا بمرض التهاب الكلى الذئبي، وتم استخدام تقنية التألق المناعي على الأنسجة المجمدة والمطمورة بالشمع البارافيني لدراسة الخزعات المأخوذة من الكلى. تم استخدام برنامج تحليل البيانات بالحاسوب لتحليل النتائج حيث كان عدد الإناث 54 (84.4%) بينما عدد الذكور 10 (15.6%) كشفت هذه الدراسة أن تألق المناعي المجمد وتألق المناعي المطمور بالشمع البارافيني (78.2%) وتألق المناعي المحمولة من الأنسجة المطمورة بالشمع البارافيني (70.3%) نقص من هذه الدراسة أنه من الممكن استخدام الأنسجة المطمورة بالشمع البارافيني بديل للأنسجة المجمدة بكفاءة عالية بعد اجراء بعض التعديلات للوصول إلى الوقت المثالي الذي يحقق نتائج ذات حساسية عالية.
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Chapter One

1. Introduction and literature review

1.1 Introduction

1.1.1 The kidneys

Are paired organs that serve several essential regulatory roles in most animals, including vertebrates and some invertebrates. They are essential in the urinary system and also serve homeostatic functions such as the regulation of electrolytes, maintenance of acid–base balance, and regulation of blood pressure (via maintaining salt and water balance). They serve the body as a natural filter of the blood, and remove wastes which are diverted to the urinary bladder. In producing urine, the kidneys excrete wastes such as urea and ammonium, and they are also responsible for the reabsorption of water, glucose, and amino acids. The kidneys also produce hormones including calcitriol, erythropoietin, and the enzyme renin.

1.1.2 Location of kidneys.

In humans the kidneys are located in the abdominal cavity, more specifically in the paravertebral gutter and lie in a retroperitoneal position at a slightly oblique angle. There are two kidneys, one on each side of the spine. The asymmetry within the abdominal cavity caused by the liver typically results in the right kidney being slightly lower than the left, and left kidney being located slightly more medial than the right. The left kidney is approximately at the vertebral level T12 to L3, and the right slightly lower, the right kidney sits just below the diaphragm and posterior to the liver, the left below the diaphragm and posterior to the spleen. Resting on top of each kidney is an adrenal gland. The upper (cranial) parts of the kidneys are partially protected by the eleventh and twelfth ribs, and each whole kidney and adrenal gland are surrounded by two layers of fat (the perirenal and pararenal fat) and the renal fascia. Each adult kidney weighs between 125 and 170 grams in males and between 115 and 155 grams in females. The left kidney is typically slightly larger than the right.

1.1.3 Development of kidney.

The development of the kidney proceeds through a series of successive phases, each marked by the development of a more advanced kidney: the pronephros, mesonephros, and metanephros. The pronephros is the most immature form of kidney, while the metanephros is most developed. The metanephros persists as the definitive adult kidney.
1.1.3.1 Pronephros.

The pronephros develops in the cervical region of the embryo. During approximately day 22 of human gestation, the paired pronephri appear towards the cranial end of the intermediate mesoderm. In this region, epithelial cells arrange themselves in a series of tubules called nephrotomes and join laterally with the pronephric duct. This duct is fully contained within the embryo and thus cannot excrete filtered material outside the embryo; therefore the pronephros is considered nonfunctional in mammals.\(^3\)

1.1.3.2 Mesonephros.

The development of the pronephric duct proceeds in a cranial-to-caudal direction. As it elongates caudally, the pronephric duct induces nearby intermediate mesoderm in the thoracolumbar area to become epithelial tubules called mesonephric tubules. Each mesonephric tubule receives a blood supply from a branch of the aorta, ending in a capillary tuft analogous to the glomerulus of the definitive nephron.\(^3\) The mesonephric tubule forms a capsule around the capillary tuft, allowing for filtration of blood. This filtrate flows through the mesonephric tubule and is drained into the continuation of the pronephric duct, now called the mesonephric duct or Wolffian duct. The nephrotomes of the pronephros degenerate while the mesonephric duct extends towards the most caudal end of the embryo, ultimately attaching to the cloaca. The mammalian mesonephros is similar to the kidneys of aquatic amphibians and fishes.\(^3\)

1.1.3.3 Metanephros.

During the fifth week of gestation, the mesonephric duct develops an outpouching, the ureteric bud, near its attachment to the cloaca. This bud, also called the metanephrogenic diverticulum, grows posteriorly and towards the head of the embryo. The elongated stalk of the ureteric bud, called the metanephric duct, later forms the ureter. As the cranial end of the bud extends into the intermediate mesoderm, it undergoes a series of branchings to form the collecting duct system of the kidney. It also forms the major and minor calyces and the renal pelvis.\(^3\) The portion of undifferentiated intermediate mesoderm in contact with the tips of the branching ureteric bud is known as the metanephrogenic blastema. Signals released from the ureteric bud induce the differentiation of the metanephrogenic blastema into the renal tubules.\(^4\) As the renal tubules grow, they come into contact and join with connecting tubules of the collecting duct system, forming a continuous passage for flow from the renal tubule to the collecting duct. Simultaneously, precursors of vascular endothelial cells begin to take their position at the tips of the renal tubules. These cells differentiate into the cells of the definitive glomerulus. In humans, all of the branches of the ureteric bud and the nephronic units have been formed by 32 to 36 weeks of gestation. However, these structures are not yet mature, and will continue to mature after birth. Once matured, humans have an estimated one million nephrons (approximately 500,000 per kidney) or more.\(^3\)(\(^4\))

1.1.3.4 Migration.

After inducing the metanephric mesenchyme the lower portions of the nephric duct will migrate caudally (downward) and connect with the bladder, thereby forming the ureters.\(^3\) The ureters will carry urine from the kidneys to the bladder for excretion.
from the fetus into the amniotic sac. As the fetus develops, the torso elongates and the kidneys rotate and migrate upwards within the abdomen which causes the length of the ureters to increase. (3)(4)

1.1.4 Histology.

Renal histology studies the structure of the kidney as viewed under a microscope. Various distinct cell types occur in the kidney, including:

- Kidney glomerulus parietal cell
- Kidney glomerulus podocyte
- Kidney proximal tubule brush border cell
- Loop of Henle thin segment cell
- Thick ascending limb cell
- Kidney distal tubule cell
- Kidney collecting duct cell
- Interstitial kidney cell

1.1.4.1 Podocyte (visceral epithelial cells)

Are cells in the Bowman’s capsule in the kidneys that wrap around the capillaries of the glomerulus. (3) The Bowman's capsule filters blood, holding back large molecules such as proteins, and passing through small molecules such as water, salts, and sugar, as the first step in forming urine. The long processes, or "foot projections," of the podocytes wrap around the capillaries, and leave slits between them. Blood is filtered through these slits, each known as a slit diaphragm or filtration slit. Several proteins are required for the foot projections to wrap around the capillaries and function. (3) When infants are born with certain defects in these proteins, such as nephrin and CD2AP, their kidneys cannot function. People have variations in these proteins, and some variations may predispose them to kidney failure later in life. Nephrin is a zipper-like protein that forms the slit diaphragm, with spaces between the teeth of the zipper, big enough to allow sugar and water through, but too small to allow proteins through. Nephron defects are responsible for congenital kidney failure. CD2AP regulates the podocyte cytoskeleton and stabilizes the slit diaphragm. (3)(6)

1.1.4.1.1 Structure.

Structural features of podocytes indicate a high rate of vesicular traffic in these cells. Many coated vesicles and coated pits can be seen along the basolateral domain of the podocytes. (3) In their cell bodies, podocytes possess a well-developed endoplasmic reticulum and a large Golgi apparatus, indicative of a high capacity for protein synthesis and post-translational modifications. There is also growing evidence of a large number of multivesicular bodies and other lysosomal components seen in these cells, indicating a high endocytic activity. "Pedicels" (or "foot processes") extend from the podocyte and increase the surface area which is crucial for the efficiency of ultrafiltration. (3)
1.1.4.1.2 Function.

Adjacent podocytes interdigitate to cover the basal lamina which is intimately associated with the glomerular capillaries, but the podocytes leave gaps or thin filtration slits. The slits are covered by slit diaphragms which are composed of a number of cell-surface proteins including nephrin, podocalyxin, and P-cadherin, which ensure that large macromolecules such as serum albumin and gamma globulin remain in the bloodstream. Small molecules such as water, glucose, and ionic salts are able to pass through the slit diaphragms and form an ultrafiltrate which is further processed by the nephron to produce urine. Podocytes are also involved in regulation of glomerular filtration rate (GFR). When podocytes contract, they cause closure of filtration slits. This decreases the GFR by reducing the surface area available for filtration.\(^4\)

1.1.4.1.3 Pathology.

Disruption of the slit diaphragms or destruction of the podocytes can lead to massive proteinuria where large amounts of protein are lost from the blood. An example of this occurs in the congenital disorder Finnish-type nephrosis, which is characterised by neonatal proteinuria leading to end-stage renal failure. This disease has been found to be caused by a mutation in the nephrin gene.\(^1\)

1.1.4.2 Proximal tubule.

The proximal tubule is the portion of the duct system of the nephron of the kidney which leads from Bowman's capsule to the loop of Henle.\(^3\) As a part of the nephron can be divided into two sections, pars convoluta and pars recta.\(^4\) Differences in cell outlines exist between these segments, and therefore presumably in function too. The "Pars convoluta" is the initial convoluted portion. In relation to the morphology of the kidney as a whole, the convoluted segments of the proximal tubules are confined entirely to the renal cortex. Some investigators on the basis of particular functional differences have divided the convoluted part into two segments designated S1 and S2. The "Pars recta" is the following straight (descending) portion. Straight segments descend into the outer medulla. They terminate at a remarkably uniform level and it is their line of termination that establishes the boundary between the inner and outer stripes of the outer zone of the renal medulla.\(^3\) The proximal tubule regulates the pH of the filtrate by exchanging hydrogen ions in the interstitium for bicarbonate ions in the filtrate; it is also responsible for secreting organic acids, such as creatinine and other bases, into the filtrate. Fluid in the filtrate entering the proximal convoluted tubule is reabsorbed into the peritubular capillaries. This is driven by sodium transport from the lumen into the blood by the Na\(^+\)/K\(^+\) ATPase in the basolateral membrane of the epithelial cells. Sodium reabsorption is primarily driven by this P-type ATPase. This is the most important transport mechanism in the PCT. Many types of medications are secreted in the proximal tubule. Most of the ammonium that is excreted in the urine is formed in the proximal tubule via the breakdown of glutamine to alpha-ketoglutarate.\(^4\) This takes place in two steps, each of which generates an ammonium anion: the conversion of glutamine to glutamate and the conversion of glutamate to alpha-ketoglutarate.\(^4\) The alpha-ketoglutarate generated in this process is then further broken down to form two bicarbonate anions, which are pumped out of the basolateral portion of the tubule cell by cotransport with sodium ions.\(^4\)

1.1.4.2.1 Structure and appearance.
The most distinctive characteristic of the proximal tubule is its brush border (or "striated border"). The luminal surface of the epithelial cells of this segment of the nephron is covered with densely packed microvilli forming a border readily visible under the light microscope giving the brush border cell its name. The microvilli greatly increase the luminal surface area of the cells, presumably facilitating their resorptive function as well as putative flow sensing within the lumen. The cytoplasm of the cells is densely packed with mitochondria, which are largely found in the basal region within the infoldings of the basal plasma membrane. The high quantity of mitochondria gives the cells an acidophilic appearance. The mitochondria are needed in order to supply the energy for the active transport of sodium ions out of the proximal tubule. Water passively follows the sodium out of the cell along its concentration gradient. Cuboidal epithelial cells lining the proximal tubule have extensive lateral interdigitations between neighboring cells, which lend an appearance of having no discrete cell margins when viewed with a light microscope. Agonal resorption of the proximal tubular contents after interruption of circulation in the capillaries surrounding the tubule often leads to disturbance of the cellular morphology of the proximal tubule cells, including the ejection of cell nuclei into the tubule lumen. This has led some observers to describe the lumen of proximal tubules as occluded or "dirty-looking," in contrast to the "clean" appearance of distal tubules, which have quite different properties.

1.1.4.3 Thin segment.

The thin segment is a segment of the nephron, which consists of: thin descending limb of loop of Henle and thin ascending limb of loop of Henle. The basement membrane of the thin limb in humans has very uniform nodular thickenings that form a network that surrounds the tubule and acts as a support structure that is homologous to the collenchyma in plants. Smith, RA et al. (Arch Pathol Lab Med Vol 108, May 1984) have designated these nodules "Belliveau Bodies" after Robert Belliveau the pathologist who originally described these structures. The epithelium is Simple squamous epithelium.

1.1.4.4 Thick segment.

The thick ascending limb of loop of Henle (TAL) also known as distal straight tubule, is a segment of the nephron in the kidney. It can be divided into two parts: that in the renal medulla, and that in the renal cortex. The medullary thick ascending limb remains impermeable to water. Sodium, potassium (K\(^+\)) and chloride (Cl\(^-\)) ions are reabsorbed by active transport. K\(^+\) is passively transported along its concentration gradient through a K\(^+\) leak channel in the apical aspect of the cells, back into the lumen of the ascending limb. This K\(^+\) "leak" generates a positive electrochemical potential difference in the lumen. This drives more paracellular reabsorption of Na\(^+\), as well as other cations such as magnesium (Mg\(^{2+}\)) and importantly calcium Ca\(^{2+}\) due to charge repulsion. The difference between the medullary and cortical thick ascending limbs is mainly anatomical. Functionally, they are very similar. The cortical thick ascending limb drains urine into the distal convoluted tubule.

1.1.4.5 Distal convoluted tubule.

The distal convoluted tubule (DCT) is a portion of kidney nephron between the loop of Henle and the collecting duct system. It is partly responsible for the regulation of potassium, sodium, calcium, and pH. It is the primary site for the kidneys' hormone
based regulation of calcium (Ca).\(^{(2)}\) On its apical surface (lumen side), cells of the DCT have a thiazide-sensitive Na-Cl cotransporter and are permeable to Ca, via TRPV5 channel. On the basolateral surface (blood) there is an ATP dependent Na/K antiport pump, a secondary active Na/Ca transporter-- antiport, and an ATP dependent Ca transporter. The basolateral ATP dependent Na/K pump produces the gradient for Na to be absorbed from the apical surface via the Na/Cl synport and for Ca to be reclaimed into the blood by the Na/Ca basolateral antiport. The DCT is lined with simple cuboidal cells that are shorter than those of the proximal convoluted tubule (PCT). The lumen appears larger in DCT than the PCT lumen because the PCT has a brush border (microvilli). DCT can be recognized by its numerous mitochondria, basal infoldings and lateral membrane interdigitations with neighboring cells. The point where DCT contacts afferent arteriole of renal corpuscle is called macula densa. It has tightly packed columnar cells which display reversed polarity and may monitor the osmolarity of blood.\(^{(2)}\)\(^{(11)}\)

### 1.1.4.6 collecting duct.

The collecting duct system of the kidney consists of a series of tubules and ducts that connect the nephrons to the ureter. It participates in electrolyte and fluid balance through reabsorption and excretion, processes regulated by the hormones aldosterone and antidiuretic hormone. There are several components of the collecting duct system, including the connecting tubules, cortical collecting ducts, and medullary collecting ducts.\(^{(4)}\)

The collecting duct system is the final component of the kidney to influence the body's electrolyte and fluid balance. In humans, the system accounts for 4–5% of the kidney's reabsorption of sodium and 5% of the kidney's reabsorption of water. At times of extreme dehydration, over 24% of the filtered water may be reabsorbed in the collecting duct system.\(^{(4)}\) The wide variation in water reabsorption levels for the collecting duct system reflects its dependence on hormonal activation. The collecting ducts, in particular, the outer medullary and cortical collecting ducts, are largely impermeable to water without the presence of antidiuretic hormone (ADH, or vasopressin). In the absence of ADH, water in the renal filtrate is left alone to enter the urine, promoting diuresis.\(^{(11)}\)\(^{(12)}\)

### 1.1.5 Structure of kidney.

![Figure 1.1 Legend](image-url)

The kidney has a bean-shaped structure; each kidney has a convex and concave surface. The concave surface, the renal hilum, is the point at which the renal artery enters the organ, and the renal vein and ureter leave. The kidney is surrounded by tough fibrous tissue, the renal capsule, which is itself surrounded by perinephric fat, renal fascia (of Gerota) and paranephric fat. The anterior (front) border of these tissues is the peritoneum, while the posterior (rear) border is the transversalis fascia. The superior border of the right kidney is adjacent to the liver; and the spleen, for the left kidney. Therefore, both move down on inhalation. The kidney is approximately 11–14 cm in length, 6 cm wide and 4 cm thick. The substance, or parenchyma, of the kidney is divided into two major structures: superficial is the renal cortex and deep is the renal medulla. Grossly, these structures take the shape of 8 to 18 cone-shaped renal lobes, each containing renal cortex surrounding a portion of medulla called a renal pyramid (of Malpighi). Between the renal pyramids are projections of cortex called renal columns (of Bertin). Nephrons, the urine-producing functional structures of the kidney, span the cortex and medulla. The initial filtering portion of a nephron is the renal corpuscle, located in the cortex, which is followed by a renal tubule that passes from the cortex deep into the medullary pyramids. Part of the renal cortex, a medullary ray is a collection of renal tubules that drain into a single collecting duct. The tip, or papilla, of each pyramid empties urine into a minor calyx; minor calyces empty into major calyces, and major calyces empty into the renal pelvis, which becomes the ureter. At the hilum, the ureter and renal vein exit the kidney while the renal artery enters. Surrounding these structures is hilar fat and lymphatic tissue with lymph nodes. The hilar fat is contiguous with a fat-filled cavity called the renal sinus. The renal sinus collectively contains the renal pelvis and calyces and separates these structures from the renal medullary tissue.

1.1.6 Function.

The kidney participates in whole-body homeostasis, regulating acid-base balance, electrolyte concentrations, extracellular fluid volume, and regulation of blood pressure. The kidney accomplishes these homeostatic functions both independently and in concert with other organs, particularly those of the endocrine system. Various endocrine hormones coordinate these endocrine functions; these include renin, angiotensin II, aldosterone, antidiuretic hormone, and atrial natriuretic peptide, among others. Many of the kidney functions are accomplished by relatively simple mechanisms of filtration, reabsorption, and secretion, which take place in the nephron. Filtration, which takes place at the renal corpuscle, is the process by which cells and large proteins are filtered from the blood to make an ultrafiltrate that eventually becomes urine. The kidney generates 180 liters of filtrate a day, while reabsorbing a large percentage, allowing for the generation of only approximately 2 liters of urine. Reabsorption is the transport of molecules from this ultrafiltrate and into the blood.
Secretion is the reverse process, in which molecules are transported in the opposite direction, from the blood into the urine.\(^2\)

### 1.1.6.1 Excretion of wastes.

The kidneys excrete a variety of waste products produced by metabolism. These include the nitrogenous wastes called "urea", from protein catabolism, as well as uric acid, from nucleic acid metabolism. Formation of urine is also the function of the kidney.\(^2\)

### 1.1.6.2 Acid base homeostasis.

Two organ systems, the kidneys and lungs, maintain acid-base homeostasis, which is the maintenance of pH around a relatively stable value. The lungs contribute to acid-base homeostasis by regulating carbonic gas (CO\(_2\)) concentration. The kidneys have two very important roles in maintaining the acid-base balance: to reabsorb bicarbonate from urine, and to excrete hydrogen ions into urine.\(^2\)

### 1.1.6.3 Osmolality regulation.

Any significant rise in plasma osmolality is detected by the hypothalamus, which communicates directly with the posterior pituitary gland. An increase in osmolality causes the gland to secrete antidiuretic hormone (ADH), resulting in water reabsorption by the kidney and an increase in urine concentration. The two factors work together to return the plasma osmolality to its normal levels. ADH binds to principal cells in the collecting duct that translocate aquaporins to the membrane, allowing water to leave the normally impermeable membrane and be reabsorbed into the body by the vasa recta, thus increasing the plasma volume of the body. \(^1\) There are two systems that create a hyperosmotic medulla and thus increase the body plasma volume: Urea recycling and the 'single effect.' Urea is usually excreted as a waste product from the kidneys. However, when plasma blood volume is low and ADH is released the aquaporins that are opened are also permeable to urea.\(^1\) This allows urea to leave the collecting duct into the medulla creating a hyperosmotic solution that 'attracts' water. Urea can then re-enter the nephron and be excreted or recycled again depending on whether ADH is still present or not. The 'Single effect' describes the fact that the ascending thick limb of the loop of Henle is not permeable to water but is permeable to NaCl. This allows for a countercurrent exchange system whereby the medulla becomes increasingly concentrated, but at the same time setting up an osmotic gradient for water to follow should the aquaporins of the collecting duct be opened by ADH.\(^1\)(\(^2\))

### 1.1.6.4 Blood pressure regulation.

Long-term regulation of blood pressure predominantly depends upon the kidney. This primarily occurs through maintenance of the extracellular fluid compartment, the
size of which depends on the plasma sodium concentration.\(^{(1)}\) Although the kidney cannot directly sense blood pressure, changes in the delivery of sodium and chloride to the distal part of the nephron alter the kidney's secretion of the enzyme renin. When the extracellular fluid compartment is expanded and blood pressure is high, the delivery of these ions is increased and renin secretion is decreased. Similarly, when the extracellular fluid compartment is contracted and blood pressure is low, sodium and chloride delivery is decreased and renin secretion is increased in response. Renin is the first in a series of important chemical messengers that comprise the renin-angiotensin system.\(^{(1)}\) Changes in renin ultimately alter the output of this system, principally the hormones angiotensin II and aldosterone. Each hormone acts via multiple mechanisms, but both increase the kidney's absorption of sodium chloride, thereby expanding the extracellular fluid compartment and raising blood pressure. When renin levels are elevated, the concentrations of angiotensin II and aldosterone increase, leading to increased sodium chloride reabsorption, expansion of the extracellular fluid compartment, and an increase in blood pressure. Conversely, when renin levels are low, angiotensin II and aldosterone levels decrease, contracting the extracellular fluid compartment, and decreasing blood pressure.\(^{(2)}\)

1.1.6.5 Hormone secretion.

The kidneys secrete a variety of hormones, including erythropoietin, and the enzyme renin. Erythropoietin is released in response to hypoxia (low levels of oxygen at tissue level) in the renal circulation. It stimulates erythropoiesis (production of red blood cells) in the bone marrow. Calcitriol, the activated form of vitamin D, promotes intestinal absorption of calcium and the renal reabsorption of phosphate. Part of the renin-angiotensin-aldosterone system, renin is an enzyme involved in the regulation of aldosterone levels.\(^{(2)}\)

1.1.7 Diseases and disorders

1.1.7.1 Congenital

- Congenital hydronephrosis
- Congenital obstruction of urinary tract
- Duplex kidneys, or double kidneys, occur in approximately 1% of the population. This occurrence normally causes no complications, but can occasionally cause urine infections.\(^{[1][2]}\)
- Duplicated ureter occurs in approximately one in 100 live births
- Horseshoe kidney occurs in approximately one in 400 live births
- Polycystic kidney disease
  - Autosomal dominant polycystic kidney disease afflicts patients later in life. Approximately one in 1000 people will develop this condition
  - Autosomal recessive polycystic kidney disease is far less common, but more severe, than the dominant condition. It is apparent in utero or at birth.
- Renal agenesis. Failure of one kidney to form occurs in approximately one in 750 live births. Failure of both kidneys to form is invariably fatal.
- Renal dysplasia
- Unilateral small kidney
- Multicystic dysplastic kidney occurs in approximately one in every 2400 live births
- Ureteropelvic Junction Obstruction or UPJO; although most cases appear congenital, some appear to be an acquired condition

### 1.1.7.2 Acquired

- Diabetic nephropathy
- Glomerulonephritis
- Hydronephrosis is the enlargement of one or both of the kidneys caused by obstruction of the flow of urine.
- Interstitial nephritis

- Kidney stones (nephrolithiasis) are a relatively common and particularly painful disorder.
- Kidney tumors
  - Wilms tumor
  - Renal cell carcinoma
- Lupus nephritis
- Minimal change disease
- In nephrotic syndrome, the glomerulus has been damaged so that a large amount of protein in the blood enters the urine. Other frequent features of the nephrotic syndrome include swelling, low serum albumin, and high cholesterol.
- Pyelonephritis is infection of the kidneys and is frequently caused by complication of a urinary tract infection.
- Renal failure
  - Acute renal failure
  - End stage kidney.

### 1.2 Literature Review

#### 1.2.1 Systemic Lupus Erythematosus (SLE)

Often abbreviated to SLE or lupus, is a systemic autoimmune disease (or autoimmune connective tissue disease) that can affect any part of the body. As occurs in other autoimmune diseases, the immune system attacks the body's cells and tissue, resulting in inflammation and tissue damage. It is a Type III hypersensitivity reaction in which antibody-immune complexes precipitate and cause a further immune response.\(^7\)

SLE most often harms the heart, joints, skin, lungs, blood vessels, liver, kidneys, and nervous system. The course of the disease is unpredictable, with periods of illness (called flares) alternating with remissions.\(^4\) The disease occurs nine times more often in women than in men, especially in women in child-bearing years ages 15 to 35, and is also more common in those of non-European descent.\(^5\)\(^6\)

There is no cure for SLE. It is treated with immunosuppression, mainly with cyclophosphamide, corticosteroids and other immunosuppressants. SLE can be fatal. Survival for people with SLE in the United States, Canada, and Europe has risen to approximately 95% at five years, 90% at 10 years, and 78% at 20 years, and now approaches that of matched controls without lupus.\(^6\)
Childhood systemic lupus erythematosus generally presents between the ages of 3 and 15, with girls outnumbering boys 4:1, and typical skin manifestations being butterfly eruption on the face and photosensitivity.\(^7\)

Lupus is Latin for wolf. In the 18th century, when lupus was just starting to be recognized as a disease, it was thought that it was caused by the bite of a wolf.\(^5\) This may have been because of the distinctive rash characteristic of lupus. (Once full-blown, the round, disk-shaped rashes heal from the inside out, leaving a bite-like imprint.)\(^7\)

The history of SLE can be divided into three periods: classical, neoclassical, and modern. The classical period began when the disease was first recognized in the Middle Ages and saw the description of the dermatological manifestation of the disorder. The term lupus is attributed to 12th-century physician Rogerius, who used it to describe the classic malar rash.\(^8\) The neoclassical period was heralded by Mór Kaposi’s recognition in 1872 of the systemic manifestations of the disease. The modern period began in 1948 with the discovery of the LE cell (the lupus erythematosus cell—a misnomer, as it occurs with other diseases as well) and is characterised by advances in our knowledge of the pathophysiology and clinical-laboratory features of the disease, as well as advances in treatment.\(^8\)

Medical historians have theorized that people with porphyria (a disease that shares many symptoms with SLE) generated folklore stories of vampires and werewolves, due to the photosensitivity, scarring, hair growth, and porphyrin brownish-red stained teeth in severe recessive forms of porphyria (or combinations of the disorder, known as dual, homozygous, or compound heterozygous porphyrias).\(^8\)

Useful medication for the disease was first found in 1894, when quinine was first reported as an effective therapy. Four years later, the use of salicylates in conjunction with quinine was noted to be of still greater benefit. This was the best available treatment until the middle of the twentieth century, when Hench discovered the efficacy of corticosteroids in the treatment of SLE.\(^9\)

### 1.2.1.1 Signs and symptoms.

SLE is one of several diseases known as “the great imitators” because it often mimics or is mistaken for other illnesses. SLE is a classical item in differential diagnosis, because SLE symptoms vary widely and come and go unpredictably. Diagnosis can thus be elusive, with some people suffering unexplained symptoms of untreated SLE for years.\(^4\)\(^10\)

Common initial and chronic complaints include fever, malaise, joint pains, myalgias, fatigue, and temporary loss of cognitive abilities. Because they are so often seen with other diseases, these signs and symptoms are not part of the diagnostic criteria for SLE. When occurring in conjunction with other signs and symptoms, however, they are considered suggestive.\(^4\)

### 1.2.1.2 Renal manifestation

Painless hematuria or proteinuria may often be the only presenting renal symptom. Acute or chronic renal impairment may develop with lupus nephritis, leading to acute or end-stage renal failure. Because of early recognition and management of SLE, end-
stage renal failure occurs in less than 5% of cases. A histological hallmark of SLE is membranous glomerulonephritis with "wire loop" abnormalities. This finding is due to immune complex deposition along the glomerular basement membrane, leading to a typical granular appearance in immunofluorescence testing.\(^6\)

1.2.1.3 Musculoskeletal manifestation

The most commonly sought medical attention is for joint pain, with the small joints of the hand and wrist usually affected, although all joints are at risk.\(^{17}\) The Lupus Foundation of America estimates more than 90 percent of those affected will experience joint and/or muscle pain at some time during the course of their illness. Unlike rheumatoid arthritis, lupus arthritis is less disabling and usually does not cause severe destruction of the joints. Fewer than ten percent of people with lupus arthritis will develop deformities of the hands and feet. SLE patients are at particular risk of developing osteoarticular tuberculosis.\(^{11}\)

A possible association between rheumatoid arthritis and SLE has been suggested,\(^{11}\) and SLE may be associated with an increased risk of bone fractures in relatively young women.\(^{12}\)

1.2.1.4 Hematological manifestation

Anemia may develop in up to 50% of cases. Low platelet and white blood cell counts may be due to the disease or a side effect of pharmacological treatment. People with SLE may have an association with antiphospholipid antibody syndrome (a thrombotic disorder), wherein autoantibodies to phospholipids are present in their serum.\(^{26}\) Abnormalities associated with antiphospholipid antibody syndrome include a paradoxical prolonged partial thromboplastin time (which usually occurs in hemorrhagic disorders) and a positive test for antiphospholipid antibodies; the combination of such findings have earned the term "lupus anticoagulant-positive". Another autoantibody finding in SLE is the anticardiolipin antibody, which can cause a false positive test for syphilis.\(^{11}\)

1.2.1.5 Cardiac manifestation

A person with SLE may have inflammation of various parts of the heart, such as pericarditis, myocarditis, and endocarditis. The endocarditis of SLE is characteristically noninfective (Libman-Sacks endocarditis), and involves either the mitral valve or the tricuspid valve. Atherosclerosis also tends to occur more often and advances more rapidly than in the general population.\(^{14}\)

1.2.1.6 Pulmonary manifestation

Lung and pleura inflammation can cause pleuritis, pleural effusion, lupus pneumonitis, chronic diffuse interstitial lung disease, pulmonary hypertension, pulmonary emboli, pulmonary hemorrhage, and shrinking lung syndrome.\(^{13}\)(\(^{14}\))

1.2.1.7 Dermatological manifestation
As many as 30% of sufferers have some dermatological symptoms (and 65% suffer such symptoms at some point), with 30% to 50% suffering from the classic malar rash (or butterfly rash) associated with the disease. Some may exhibit thick, red scaly patches on the skin (referred to as discoid lupus). Alopecia; mouth, nasal, urinary tract and vaginal ulcers, and lesions on the skin are also possible manifestations. Tiny tears in delicate tissue around the eyes can occur after even minimal rubbing.\(^{(4)}\)

### 1.2.1.8 Neuropsychiatric manifestation

Neuropsychiatric syndromes can result when SLE affects the central or peripheral nervous systems. The American College of Rheumatology defines 19 neuropsychiatric syndromes in systemic lupus erythematosus. The diagnosis of neuropsychiatric syndromes concurrent with SLE is one of the most difficult challenges in medicine, because it can involve so many different patterns of symptoms, some of which may be mistaken for signs of infectious disease or stroke.\(^{(15)}\)

The most common neuropsychiatric disorder people with SLE have is headache, although the existence of a specific lupus headache and the optimal approach to headache in SLE cases remains controversial. Other common neuropsychiatric manifestation of SLE include cognitive dysfunction, mood disorder, cerebrovascular disease, seizures, polyneuropathy, anxiety disorder, and psychosis. It can rarely present with intracranial hypertension syndrome, characterized by an elevated intracranial pressure, papilledema, and headache with occasional abducens nerve paresis, absence of a space-occupying lesion or ventricular enlargement, and normal cerebrospinal fluid chemical and hematological constituents.\(^{(16)}\)

More rare manifestations are acute confusional state, Guillain-Barré syndrome, aseptic meningitis, autonomic disorder, demyelinating syndrome, mononeuropathy (which might manifest as mononeuritis multiplex), movement disorder (more specifically, chorea), myasthenia gravis, myelopathy, cranial neuropathy and plexopathy.\(^{(16)}\) Neural symptoms contribute to a significant percentage of morbidity and mortality in patients with lupus. As a result, the neural side of lupus is being studied in hopes of reducing morbidity and mortality rates. The neural manifestation of lupus is known as neuropsychiatric systemic lupus erythematosus (NPSLE). One aspect of this disease is severe damage to the epithelial cells of the blood–brain barrier.\(^{(17)}\) Lupus has a wide range of symptoms which span the body. The neurological symptoms include headaches, depression, seizures, cognitive dysfunction, mood disorder, cerebrovascular disease, polyneuropathy, anxiety disorder, psychosis, and in some extreme cases, personality disorders. In certain regions, depression reportedly affects up to 60% of women suffering from SLE.\(^{(17)}\)

### 1.2.1.9 Reproductive disorders

SLE causes an increased rate of fetal death in utero and spontaneous abortion (miscarriage). The overall live-birth rate in SLE patients has been estimated to be 72%. Pregnancy outcome appears to be worse in SLE patients whose disease flares up during pregnancy.\(^{(18)}\) Neonatal lupus is the occurrence of SLE symptoms in an infant born from a mother with SLE, most commonly presenting with a rash resembling discoid lupus erythematosus, and sometimes with systemic abnormalities such as
heart block or hepatosplenomegaly. Neonatal lupus is usually benign and self-limited.\(^{(18)}\)

### 1.2.1.10 Pathophysiology

In SLE, the body's immune system produces antibodies against itself, particularly against proteins in the cell nucleus. SLE is triggered by environmental factors that are unknown. In order to preserve homeostasis, the immune system must balance between being sensitive enough to protect against infection, and becoming sensitized to attack the body's own proteins (autoimmunity).\(^{(19)}\) During an immune reaction to a foreign stimulus, such as bacteria, virus, or allergen, immune cells that would normally be deactivated due to their affinity for self tissues can be abnormally activated by signaling sequences of antigen-presenting cells.\(^{(19)}\) Thus triggers may include viruses, bacteria, allergens (both IgE and hypersensitivity), and can be aggravated by environmental stimulants such as ultraviolet light and certain drug reactions. These stimuli begin a reaction that leads to destruction of other cells in the body and exposure of their DNA, histones, and other proteins, particularly parts of the cell nucleus. The body's sensitized B-lymphocyte cells will now produce antibodies against these nuclear-related proteins. These antibodies clump into antibody-protein complexes which stick to surfaces and damage blood vessels in critical areas of the body, such as the glomeruli of the kidney; these antibody attacks are the cause of SLE. Researchers are now identifying the individual genes, the proteins they produce, and their role in the immune system. Each protein is a link on the autoimmune chain, and researchers are trying to find drugs to break each of those links. SLE is a chronic inflammatory disease believed to be a type III hypersensitivity response with potential type II involvement. Reticulate and stellate acral pigmentation should be considered a possible manifestation of SLE and high titers of anticardiolipin antibodies, or a consequence of therapy.\(^{(19)}\)

### 1.2.2 Lupus Nephritis

Systemic lupus nephritis (SLE) is a multisystemic disease in which renal involvement is one of the most serious complications. The disease may occur at any age, but is most common between the age of 15 and 40 years, with a female preponderance of 4 to 1. The characteristic pattern of the syndrome is the great variability of clinical symptoms; joint and dermal manifestations are the most frequent.\(^{(13)}\) Fever, fatigability, weight loss, lymphadenopathy and occasionally recurrent thrombophlebitis may be the initial warning signs. Autoimmune haematological disorders such as haemolytic anaemia leucopenia and thrombocytopenia are seen in nearly all patients during the course of the disease. Serological abnormalities such as false positive serologic test for syphilis, the presence of a wide variety of antibodies to autologous tissue, plasma antigens and low serum complement activity appears during the active stage of the disease.\(^{(13)}\)(8)
The incidence of renal disease in SLE is high, ranging from 50 to 80 percent in several large series of cases. Renal involvement may occur at any time during the course of SLE, but in most patients develops during the first two years, and rarely it may be the first manifestation of SLE. Morbidity and mortality are nearly twice as great if the onset is early and the patient is young. Pregnancy, particularly during the first trimester or during eight weeks postpartum, may precipitate the development or induce exacerbation of SLE.

The clinical spectrum of renal involvement ranges from asymptomatic microscopic haematuria and mild proteinuria to nephritic syndrome, renal insufficiency, hypertension and uremia. Proteinuria alone or associated with microscopic haematuria is the most common finding. Urinary sediment abnormalities vary accordingly; they include: red blood cells often occurring in clumps, suggesting a diagnosis of pyelonephritis, double refractile and oval fat bodies, and "telescoped" sediment containing all types of cells, formed elements, and casts reflecting damage to the entire nephrons.

Nephrotic syndrome with heavy proteinuria develops during the course of the disease in many patients with SLE. The nephritic syndrome is characterized by the absence or late development of hyperlipidaemia. A high proportion of patients develop renal insufficiency, but the advent of high doses of steroids and immunosuppressive therapy have reduced the mortality. Hypertension is rarely seen early in the disease and roughly parallels the severity of renal damage.

The pathologic mechanism for glomerular damage in lupus nephritis is the deposition of immune complexes in glomeruli, demonstrable by Immunofluorescence as irregular lumpy or granular deposits, corresponding to dense granular deposits seen by electron microscopy. IgG, IgM and IgA are present in granular distribution. In addition, at times, fibrinogen is found in glomerular lesions of active lupus nephritis, indicating that the coagulation process may be another or an additional, pathogenic mechanism in glomerular injury. The recent demonstration of NDNA, as well as SDNA, and anti-SDNA in glomerular deposits in a similar pattern to that of gamma globulin and complement, provides evidence that autologous immune complexes play a role in the pathogenesis of the renal disease in systemic lupus erythematosus.

As clinical renal symptoms vary, so do morphologic renal changes. The main renal changes are confined to the glomeruli. Interstitial changes often parallel the extent of glomerular disease. Tubular and vascular changes are secondary.

### 1.2.2.1 Etiology of SLE

SLE is a multisystem autoimmune disease whose etiology and pathogenesis are incompletely understood. The development of autoimmunity in SLE has been attributed to a loss of self-tolerance due to inadequate central or peripheral deletion or silencing of autoreactive lymphocytes, leading to multiple autoantibody specificities. Dysregulated apoptosis and inadequate removal of apoptotic cells and nuclear remnants may contribute to autoimmunity by causing prolonged exposure of
the immune system to nuclear and cell membrane component. The characteristic
development of autoantibodies to DNA and other nuclear antigens, as well as to
membrane phospholipids, support the relevance of both mechanisms. In addition to
established genetic predisposition, altered immunoregulatory factors or environmental
stimuli may trigger autoimmune phenomena in certain populations. Recent studies
have ascribed specific genetic linkages to the development of renal disease in SLE
among certain ethnic groups, including European American and African American
populations, some of which may determine the severity of the glomerular
disease. 

1.2.2.2 Pathogenesis of Tissue Injury in SLE

Although knowledge of the etiology of SLE is incomplete, it is clear from the
variably forms of tissue injury that a number of different effector mechanisms may act
alone or in concert to produce the pleomorphic patterns of lupus nephritis.

Autoantibodies may lead to cell and tissue injury by Fc receptor-mediated
inflammation as well as by direct cytotoxicity, which is usually complement-
dependent, as has been shown for antibody-mediated hemolytic anemia or
thrombocytopenia. In the kidney, intrinsic antigens such as extracellular matrix
components or cell surface glycoproteins may serve as targets for autoantibody
binding. In addition, renal injury in lupus nephritis may result from autoantibodies
that bind to circulating antigens, forming circulating preformed immune complexes,
or autoantibodies that bind to antigens deposited from the circulation in glomerular
and vessel walls, causing in situ immune complex formation, as has been shown for
nucleosomes and antidouble-stranded DNA autoantibodies. Subsequent Fc receptor
and complement binding then initiates an inflammatory and cytotoxic reaction. Such
cytotoxicity may be directed toward podocytes in the setting of membranous
nephropathy, where in situ immune complex formation occurs along the subepithelial
aspect of the glomerular basement membrane, or toward endocapillary cells in the
case of the endocapillary proliferative and exudative inflammatory reaction that
follows subendothelial immune complex formation. 

In addition to direct immune complex-mediated cell and tissue injury, autoantibodies
with antiphospholipid or cryoglobulin activity also promote thrombotic and
inflammatory vascular lesions in SLE. Antineutrophil cytoplasmic-antigen
autoantibodies (ANCA) have been described in a subgroup of patients with lupus
nephritis and may initiate vasculitis and glomerulonephritis by “pauci-immune”
neutrophil-dependent mechanisms similar to those described for microscopic
polyangiitis or Wegener’s granulomatosis. Finally, it is also likely that other poorly
characterized autoantibodies of unknown specificity (such as anti-endothelial
antibodies) may be operant in the pathogenesis of some forms of lupus nephritis.

1.2.2.3 Glomerular Patterns of Injury

Based on various experimental models of autoimmune and immune complex
disease in the kidney and on observations in human renal biopsies, it is now well
established that the glomerular patterns of immune complex-mediated injury are
related to the site of accumulation of immunoglobulins, their antigen specificity, their
capacity to bind and activate complement and other serine proteases, and their ability
to evoke a cellular inflammatory response. These patterns of injury can be divided
into three groups.
1.2.2.3.1 Mesangial Pattern

In the mesangial pattern, mesangial hypercellularity and matrix accumulation result from mesangial immune complex accumulation, as can occur in IgA nephropathy or in mesangial proliferative lupus nephritis.\(^\text{18}\)

1.2.2.3.2 Endothelial Pattern

In the endothelial pattern, an exudative component characterized by leukocyte accumulation, endothelial cell injury, and endocapillary proliferation. This pattern is often associated with capillary wall destruction, mild to marked immune complex deposition, and varying degrees of mesangial proliferation and crescent formation. This category is exemplified by severe postinfectious glomerulonephritis, antiglomerular basement membrane (GBM) disease, systemic vasculitis, and endocapillary proliferative forms of lupus glomerulonephritis, for example. Within the endothelial pattern of glomerular injury, a diffuse and global form can often be separated from a focal segmental form (as seen in microscopic polyangiitis), in which different pathogenetic mechanisms may prevail. The endothelial pattern of injury can also be caused by nonimmunologic mechanisms, such as shear-stress in malignant hypertension, bacterial toxins in verocytotoxin-induced thrombotic microangiopathy, and thrombotic events in SLE-associated lupus anticoagulant syndrome.\(^\text{18}\) Persistent accumulation of immune complexes in the subendothelial space may lead to more severe injury and chronic changes, including cellular interposition and replication of the GBM. These endocapillary changes usually occur in association with mesangial pathology because the mesangium is in direct continuity with the subendothelial space and is accessible to circulating immune complexes. This combined mesangiocapillary or membranoproliferative pattern of injury is particularly common in the chronic phase of lupus nephritis.\(^\text{18}\)

1.2.2.3.3 Epithelial Pattern

In the epithelial pattern, antibodies and complement inflict cytotoxic injury on the podocyte resulting in a nonexudative, nonproliferative capillary wall lesion, as can be seen in idiopathic and SLE-associated forms of membranous glomerulopathy.\(^\text{17}\)

The usual clinical manifestations of these three major morphologic patterns can be predicted based on the topography and character of the glomerular lesions. Mesangial pathology leads to a syndrome of microscopic hematuria and subnephrotic proteinuria with well-preserved or minimally reduced glomerular filtration rate (GFR); the endocapillary pattern is characterized by an acute reduction in GFR, hematuria, and mild to moderate proteinuria; and the membranous pattern is associated with significant proteinuria, often with nephrotic syndrome, and with preservation or gradual reduction in GFR. These three patterns of injury, which encompass the spectrum of most glomerular diseases regardless of etiology, also apply to the major subtypes of glomerular involvement in SLE. In lupus glomerulonephritis, as in other glomerular diseases, it is not uncommon for several different morphologic patterns to coexist, leading to a more complex clinical expression of disease.\(^\text{17,18}\)
1.2.3 Classification of Lupus Nephritis: History

The introduction of renal biopsy in the 1950s, the application of immunofluorescence and electron microscopic techniques in the 1960s, and increasing knowledge about mechanisms of immune-mediated glomerular injury derived from experimental studies on serum sickness and other models formed the basis of the recognition and classification of the various patterns of renal injury in SLE. As early as 1964, focal segmental glomerulitis, diffuse proliferative glomerulonephritis, and membranous glomerulopathy were recognized as separate entities, followed by the identification of mesangial lesions in the 1970s. (19)

The first World Health Organization (WHO) classification was formulated by Pirani and Pollak in Buffalo, New York in 1974 and was first used in publications in 1975 and 1978. This classification addressed glomerular lesions only. Class I was applied to renal biopsies showing no detectable glomerular abnormalities by light, fluorescence, or electron microscopy. Class II was defined as purely mesangial immune deposition and was subdivided into two subclasses depending on whether mesangial hypercellularity was present. Class III lesions were defined as proliferative glomerulonephritis affecting fewer than 50% of the glomeruli, whereas class IV was defined as proliferative glomerulonephritis affecting more than 50% of the glomeruli. (33) No qualitative differences between class III and class IV lesions were described. Membranous lupus nephritis was classified as class V. Tubulointerstitial and vascular lesions were not included in the classification system. (30)

In 1982, the WHO classification was modified by the International Study of Kidney Diseases in Children. Class I was applied to normocellular glomeruli and was now divided into two subclasses based on whether mesangial immune deposits were identified. Class II was applied to purely mesangial proliferative glomerulonephritis and was divided into two subcategories based on the severity of the mesangial hypercellularity. Class III now denoted focal segmental glomerulonephritis with necrotizing lesions and class IV was used for diffuse glomerulonephritis, without stipulating criteria for the percentage of affected glomeruli. Within class IV, there were subdivisions of variants with severe mesangial proliferation, membranoproliferative features, or extensive subendothelial immune deposits in the absence of endocapillary proliferation. (21) In addition, the 1982 classification introduced subdivisions for class III and IV based on the presence of active, chronic, or mixed types of glomerular injury. Class V denoted membranous glomerulonephritis but was subdivided based on the presence of mesangial hypercellularity and overlaps with focal proliferative (class III) and diffuse proliferative (class IV) lupus nephritis. Class VI was introduced to denote advanced sclerosing glomerulonephritis, although the percentage of glomeruli requiring sclerosis was not stipulated. The use of numerous subcategories and the handling of mixed classes made this modified classification cumbersome for some pathologists to use and impeded effective communication with the clinicians. These drawbacks prompted many pathologists to continue to work with the older 1974 WHO classification. (21)(22)

1.2.4 Classification of Lupus Nephritis: New Proposal by WHO

In order to accommodate the clinicopathologic and pathogenetic insights that have accumulated since the 1982 and 1995 modifications of the original 1974 WHO classification and to eliminate inconsistencies and ambiguities, WHO propose a new revised classification. (36) This revised classification preserves the simplicity of the original WHO classification, incorporates selective refinements concerning activity
and chronicity from the 1982 and 1995 revisions, and adds a number of new modifications. Overall, it bears a strong similarity to the 1974 classification, but introduces several important modifications concerning quantitative and qualitative differences between class III and IV lesions. The major objective is to standardize definitions, emphasize clinically relevant lesions, and encourage uniform and reproducible reporting between centers. Like the preceding classifications, this new classification is based exclusively on glomerular pathology. WHO strongly recommend that any significant vascular and tubulointerstitial pathology be reported as separate entries in the diagnostic line. (23)

As a premise, WHO emphasize that adequacy of the tissue specimen and histopathologic techniques are mandatory for a reliable classification. For accurate pathologic analysis, it is important that the tissue should be optimally preserved, processed by a skilled technician, cut at 3 microns, and sectioned at multiple levels. Proper tissue handling and use of special stains are essential for accurate and complete assessment of glomerular number, cellularity, and capillary wall alterations. (23)

In order to reasonably exclude a focal lesion, the biopsy should contain a minimum of 10 glomeruli for light microscopic analysis. Immunofluorescence is required for complete renal biopsy analysis and should include staining for IgG, IgA, and IgM isotypes, kappa and lambda light chains, and complement components C3 and C1q. Glomerular immune deposits attributable to lupus nephritis as detected by immunofluorescence almost always contain dominant polyclonal IgG, as well as C3 and in most instances C1q, with variable codeposits of IgA and IgM. If glomerular immunoglobulin deposits are restricted to IgA or IgM, diagnostic possibilities other than lupus nephritis should be considered in correlation with serologic and clinical findings. (23)

While the role of electron microscopy in the diagnosis and classification of lupus glomerulonephritis cannot be underestimated and may be essential in some cases, the lack of readily available electron microscopy facilities in many centers throughout the world should not prevent the skilled pathologist from rendering a diagnosis of lupus nephritis using a combination of complete light microscopic and immunofluorescence studies. We recommend appropriate fixation and storage of a sample of renal cortical tissue for ultrastructural evaluation when needed. (23)(24)

1.2.4.1 Class I

Class I is defined as minimal mesangial lupus nephritis with mesangial accumulation of immune complexes identified by immunofluorescence, or by immunofluorescence and electron microscopy, without concomitant light microscopic alterations. A complete lack of renal abnormalities by light microscopy, immunofluorescence, and electron microscopy no longer qualifies as class I, and in this respect is a change from the 1974 WHO classification. (23)(24)

1.2.4.2 Class II

Class II is defined as mesangial proliferative lupus nephritis characterized by any degree of mesangial hypercellularity (defined as three or more mesangial cells per mesangial area in a 3 micron thick section) in association with mesangial immune deposits. By immunofluorescence or electron microscopy, there may be rare isolated small immune deposits involving the peripheral capillary walls in some examples of class II. (36) However, the identification of any subendothelial deposits by light
microscopy would warrant a designation of class III or class IV depending on the extent and distribution of the subendothelial deposits. Similarly, the presence of any global or segmental glomerular scars that are interpreted as the sequela of previous glomerular endocapillary proliferation, necrosis or crescents is incompatible with class II and would be consistent with either class III or class IV depending on the number of scarred glomeruli. (23)(24)

1.2.4.3 Class III

Class III is defined as focal lupus nephritis involving less than 50% of all glomeruli. Affected glomeruli usually display segmental endocapillary proliferative lesions or inactive glomerular scars, with or without capillary wall necrosis and crescents, with subendothelial deposits (usually in a segmental distribution). In assessing the extent of the lesions, glomeruli with both active and sclerotic lesions will be taken into account. Focal or diffuse mesangial alterations (including mesangial proliferation or mesangial immune deposits) may accompany the focal glomerular lesions. In a pilot study of pathologists from seven different centers on 50 consecutive cases of lupus glomerulonephritis, for a total of 350 specimens, class III lesions were found to be almost invariably segmental and rarely global. Vasculitis-like lesions characterized by segmental capillary necrosis in the absence of endocapillary proliferation were rare. (23)(24)

1.2.4.4 Class IV

Class IV is defined as diffuse lupus nephritis involving 50% or more of glomeruli in the biopsy. In the affected glomeruli, the lesions as described below may be segmental, defined as sparing at least half of the glomerular tuft, or global, defined as involving more than half of the glomerular tuft. This class is subdivided into diffuse segmental lupus nephritis (class IV-S) when >50% of the involved glomeruli have segmental lesions, and diffuse global lupus nephritis (class IV-G) when >50% of the involved glomeruli have global lesions. Class IV-S typically shows segmental endocapillary proliferation encroaching upon capillary lumina with or without necrosis, and may be superimposed upon similarly distributed glomerular scars. Class IV-G is characterized by diffuse and global endocapillary, extracapillary, or mesangiocapillary proliferation or widespread wireloops. Any active lesion may be seen with class IV-G, including karyorrhexis, capillary loop necrosis, and crescent formation. Rare examples of extensive (diffuse and global) subendothelial glomerular deposits with little or no proliferation should also be included in this category. The new subdivision for segmental and global lesions is based on evidence suggesting that diffuse segmental lupus nephritis may have a different outcome than diffuse global lupus nephritis. In the pilot study of seven different centers mentioned above, 35% of 135 class IV biopsies revealed a predominantly segmental distribution of lesions, as opposed to 65% that showed a predominantly global distribution. The study further showed that fibrinoid necrosis is usually associated with endocapillary hypercellularity and may therefore be a more severe expression of the same pathogenetic mechanism. (23)(24)
In the report, parameters of activity and chronicity should be described. In the diagnostic line, the proportion of glomeruli affected by active and chronic lesions and by fibrinoid necrosis or crescents should be indicated. In addition, the presence of any tubulointerstitial or vascular pathology should be reported in the diagnostic line. (23)(24)

It is recognized that scattered subepithelial deposits are commonly seen in class IV biopsies. Therefore, a diagnosis of combined class IV and class V is warranted only if subepithelial deposits involve at least 50% of the glomerular capillary surface area in at least 50% of glomeruli by light microscopy or immunofluorescence microscopy. (23)(24)

In assessing the extent of the lesions, both active and sclerotic lesions will be taken into account. By way of illustration, a renal biopsy containing a total of 20 glomeruli, of which there are segmental active proliferative lesions in four and segmental inactive scarred lesions in ten should be designated class IV-S lupus nephritis. (23)(24)

1.2.4.5 Class V

Class V is defined as membranous lupus nephritis with global or segmental continuous granular subepithelial immune deposits, often with concomitant mesangial immune deposits. Any degree of mesangial hypercellularity may occur in class V. Scattered subendothelial immune deposits may be identified by immunofluorescence or electron microscopy. If present by light microscopy, subendothelial deposits warrant a combined diagnosis of lupus nephritis class III and V, or class IV and V, depending on their distribution. When a diffusely distributed membranous lesion (involving >50% of the tuft of >50% of the glomeruli by light microscopy or immunofluorescence) is associated with an active lesion of class III or IV, both diagnoses are to be reported in the diagnostic line. As class V evolves to chronicity, there is typically the development of segmental or global glomerulosclerosis, without the superimposition of proliferative lupus nephritis. However, if the glomerular scars are judged to be the sequela of previous proliferative, necrotizing or crescentic glomerular lesions, then a combined designation of class III and class V lupus nephritis, or class IV and class V lupus nephritis should be applied, depending on the distribution of the glomerular scarring. (23)(24)

1.2.4.6 Class VI

Class VI (advanced-stage lupus nephritis) designates those biopsies with ≥90% global glomerulosclerosis and in which there is clinical or pathologic evidence that the sclerosis is attributable to lupus nephritis. There should be no evidence of ongoing active glomerular disease. Class VI may represent the advanced stage of chronic class III, class IV, or class V lupus nephritis. Without the aid of sequential renal biopsies, it may be impossible to determine from which class the sclerotic glomerular lesions evolved. (23)(24)

By light microscopy, glomerular changes vary from mild focal to wide-spread and extensive but, almost invariably, the focal character and the pleomorphism of the lesions are retained. All possible reactions involving each of the glomerular component may occur, they include minimal changes confined only to the mesangium, cellular proliferation, leucocytic infiltration, basement membrane changes fibrinoid necrosis  nuclear fragmentation and capillary thrombosis and crescent formation. (25) Proliferation of mesangial endothelial and to a lesser degree parietal epithelial cells may be localized usually confined to a peripheral lobule, or
extensive, but it is always irregular. The proliferative lesions are commonly associated with basement membrane thickening which in most cases is focal involving few or several peripheral loops. This basement membrane thickening may be mild or marked and has a refractile quality referred to as "wire looping." The term fibrinoid necrosis is applied to the amorphous, brightly eosinophilic background in an area of hypercellularity, obliterating capillary lumina. Pyknotic nuclei, fragmented nuclear debris and at times, haematoxylin bodies are also seen in this area. Haematoxylin bodies are oval or rounded, purplish structures found within areas of fibrinoid necrosis or occasionally in capillary lumina. Although pathognomonic they are often absent in biopsy material. (38) Intercapillary "hyaline" thrombi appear as amorphous pink material occluding capillary lumina. In some cases the only change is diffuse basement membrane thickening indistinguishable from that seen in idiopathic membranous glomerulonephritis but for the focal increase in mesangial cells. (25)

By electron microscopy the most conspicuous findings are the coarse granular electron-dense deposits. They may be present in various locations—mesangial, subendothelial, intramembranous, and subepithelial. Subendothelial deposits correlate with low serum complement levels and active renal disease. Massive subendothelial, circumferential deposits correspond to the wire looping seen by light microscopy. In some cases subepithelial deposits and basement membrane spiking indistinguishable from idiopathic membranous glomerulonephritis dominate the picture. (25)

In most patient with SLE micro tubular inclusions are seen within the endothelial cell cytoplasm of glomerular and interstitial capillaries therefore their presence is diagnostically helpful. The supposition that they represent virus particles could not be substantiated and these are now regarded by most investigators as modifications of endoplasmic reticulum in response to injury. (38)

The changes interpreted by light microscopy as fibrinoid necrosis and hyaline thrombosis are found to be massive deposits by electron microscopy. (38) Intracapillary fibrin is an uncommon electron microscopic finding and true cellular necrosis is rare. At times strands of fibrin can be identified in Bowman's space in association with epithelial crescent. Visceral epithelial cells may show swelling increase in endoplasmic reticulum organelles, dense bodies and fusion of foot processes overlying deposits. At times granular electron-dense deposits are found in arteriolar walls around intertubular capillaries and in tubular basement membranes. (25)

Similary Immunoresponse studies show marked variation in location and size of deposits of immunoglobulins and complement. (25)

On the basis of morphologic findings lupus nephritis can be divided into four major groups: mesangial, focal proliferative, diffuse proliferative and membranous lupus nephritis. (25)

In mesangial lupus nephritis mesangial accentuation with mild increase in cells and matrix is the only finding by light microscope. By electron microscope the deposits are confined to the mesangium. By Immunoresponse microscopy mesangial deposits of IgG complement and occasionally IgA are present. (25)
In focal proliferative lupus nephritis only a number (less than 50%) of the glomeruli show local involvement. The remaining glomeruli appear normal and the tubular and interstitial changes are mild. By electron microscopy electron-dense deposit are seen mainly in the mesangium, but also beneath the endothelium or in various locations in relation to the basement membrane. Most of the deposits are discete and their distribution focal. By Immunofluorescence microscopy focal granular and rarely lumpy deposits of gamma globulins and complement are found in the mesangium and along the capillary wall. The clinical features in patients with focal lupus nephritis are usually mild proteinuria and haematuria but more severe renal manifestations appear during the active phase of the disease and subside with spontaneous or therapeutically induced remission. Patient may survive for many years and persistent nephritic syndrome, renal insufficiency and hypertension are uncommon.

Diffuse proliferative lupus nephritis is characterized by widespread glomerular involvement. Frequently the lesions are irregular and pleomorphic but exceptionally may appear fairy uniform. The entire nephrons is affected and tubular degeneration and atrophy, irregular interstitial inflammatory cell infiltration and rarely vascular changes are present. By electron and fluorescence microscopy the distribution of deposits is similar but more extensive. In addition fibrin may also be present.

Patients with this type of change have clinical evidence of active renal disease often characterized by nephritic syndrome renal insufficiency or both. The degree of histologic change and the amount and size of deposits particularly subendothelial roughly correlate with renal function status. The course is that of progressive severe renal disease, and death may occur within months but with modern therapy remissions are reported with greater frequency.

The differentiation between focal and diffuse proliferative lupus nephritis is arbitrary. If 10 to 50 percent of glomeruli present in biopsy are involved the disease is classified as focal. The ultrastructural and Immunofluorescence finding may be similar in both and vary only in the extent of involvement.

Membranous lupus nephritis is the least common form. The light and fluorescence microscopic changes are similar or identical to those seen in idiopathic membranous glomerulonephritis. By light microscopy diffuse basement membrane thickening and spiking in PAMS stained section are seen. By fluorescence microscopy granular deposit of immunoglobulins and complement are present along the basement membrane. By electron microscopy in addition to the epimembranous deposits mesangial deposits and microtubular endothelial, cytoplasmic inclusion are at times present and helpful in differential diagnosis.

Clinically the patient presents with heavy proteinuria or nephritic syndrome associated with microscopic haematuria. The patient's course is prolonged. Spontaneous or induced remissions of nephritic syndrome may occur but proteinuria usually persist while some patient may developed renal insufficiency. Hypertension and spicaemia are common late complication.
Thus four different forms of lupus nephritis can be recognized on the basis of light, electron and fluorescence microscopy. Although this classification is arbitrary and at times overlapping it correlates to some extent with the clinical features and course of the disease.\(^{(25)(26)}\)

The clinical symptoms correlate best with the presence or absence of active lesion rather than the number of glomeruli involved. Although in many patients the renal lesion may remain in one of the four morphologic forms for a long period, transformation from one form to another may occur in all forms. In addition the treatment may influence the morphology and changes from diffuse to focal or from diffuse to membranous are not unusual.\(^{(25)}\)

### 1.2.5 Antigen Antibody Interaction

#### 1.2.5.1 Antigen

An antigen is a substance that when introduced into the body induces a specific immune response. Antigens include toxins, drugs, parasites, viruses, bacteria, foreign blood cells and cells of transplanted organs. Some times the body own protein expressed in an inappropriate manner is treated like antigen by the immune system and the result is autoimmune disease.\(^{(32)}\) Antigens are recognized by B cells and their surface antibody or by T cell receptors. Recognition leads to the growth of specific clones of B cell and T cell which recognized the antigen. Each part of the antigen that is recognized by B cells or T cell receptors is known as antigen epitope depending on the size of protein, glycoprotein or polysaccharides. There may be hundreds of B cell epitope (recognized by different antibody) or T cell epitope (presented by antigen presenting cell to different T cell) in the same molecule.\(^{(32)}\)

#### 1.2.5.2 Antibodies

Also called immunoglobulins are one of two important protein molecules of the immune system that engage in the recognition of pathogens or other foreign materials. This process is called antigen recognition and is a pivotal process in the immune response. The other antigen recognition molecule is found on T cell and is called T cell receptor. Antibodies act as recognition units on the surface of B cell. There are different classes (Isotopes) of immunoglobulin IgD, IgA, IgM, IgG, IgE.\(^{(32)}\) There are sub classes of the five classes and they vary among species. The ability of antibody to recognize specific antigen is an important characteristic. Antigen recognition and binding allows antibody to perform four important effectors functions: Opsonization for phagocytosis, activating complement, neutralizing toxins and blocking attachment pathogens to cell or tissue.\(^{(32)}\) An antibody is made up of four polypeptides (chain), two heavy chain and two light chain in each antibody. Heavy chain and light chain are held together by disulphide bonds. In human there are two different kinds of light chain (kappa and lambda) and five kinds of heavy chain (m,d,g,e,a). The two important regions of the antibody are Fab region and Fc region. The antigen binding region Fab is the recognition region of the antibody, it has great diversity. The Fc region is responsible for the effector function of the antibody such as opsonization,
complement activation and also define the class of antibody IgA, IgG, IgM, IgD, IgE, and their subclasses.\(^{(32)}\)

### 1.2.5.3 Antigen antibody reaction

The antibodies on the surface of B cells and the soluble antibodies in the blood and tissues recognize antigens in the native form. This means that antibodies can recognize antigen on the surface of bacteria or viruses as well as antigen free-floating in the tissues (for example, bacterial toxins). For example, an HIV-infected person will develop a vigorous antibody response to the gp120 glycoprotein on the surface of the HIV virus. Antibodies of this type help prevent viral spread by blocking attachment of viruses to their target cells and are often called “neutralizing” antibodies.\(^{(32)}\)

In addition to interacting with antigen on the surface of pathogens, antibodies can also interact with free antigen in the blood or tissues. This antigen is usually released by the pathogen or the result of pathogen lysis by the other immune components. Antibody binds to this free antigen and creates antigen-antibody complexes (immune complexes) of various sizes. Most immune complexes are taken out of circulation in the liver by phagocytic cells but some can be deposited in tissues and initiate inflammatory responses which can lead to significant tissue pathology and chronic inflammatory conditions.\(^{(32)}\)

### 1.2.6 Complement system

In the late 19th century, Hans Ernst August Buchner found that blood serum contained a "factor" or "principle" capable of killing bacteria. In 1896, Jules Bordet, a young Belgian scientist in Paris at the Pasteur Institute, demonstrated that this principle had two components: one that maintained this effect after being heated, and one that lost this effect after being heated. The heat-stable component was responsible for the immunity against specific microorganisms, whereas the heat-sensitive (heat-labile) component was responsible for the non-specific antimicrobial activity conferred by normal serum.\(^{(18)}\) This heat-labile component is what we now call "complement" earlier known as "alexine". The term "complement" was introduced by Paul Ehrlich in the late 1890s, as part of his larger theory of the immune system. According to this theory, the immune system consists of cells that have specific receptors on their surface to recognize antigens.\(^{(18)}\) Upon immunisation with an antigen, more of these receptors are formed, and they are then shed from the cells to circulate in the blood. These receptors, which we now call "antibodies," were called by Ehrlich "amboceptors" to emphasise their bifunctional binding capacity: They recognise and bind to a specific antigen, but they also recognise and bind to the heat-labile antimicrobial component of fresh serum. Ehrlich, therefore, named this heat-labile component "complement," because it is something in the blood that "complements" the cells of the immune system.\(^{(35)}\) In the early half of the 1930s, a team led by the renowned Irish researcher, Jackie Stanley, stumbled upon the all-important opsonisation-mediated effect of C3b. Building off Ehrlich's work, Stanley's team proved the role of complement in both the innate as well as the cell-mediated immune response.\(^{(35)}\)

Ehrlich believed that each antigen-specific amboceptor has its own specific complement, whereas Bordet believed that there is only one type of complement. In the early 20th century, this controversy was resolved when it became understood that
complement can act in combination with specific antibodies, or on its own in a non-specific way.\(^{(35)}\)

The proteins and glycoproteins that constitute the complement system are synthesized by the liver hepatocytes. But significant amounts are also produced by tissue macrophages, blood monocytes, and epithelial cells of the genitourinal tract and gastrointestinal tract. The three pathways of activation all generate homologous variants of the protease C3-convertase.\(^{(35)}\) The classical complement pathway typically requires antigen:antibody complexes for activation (specific immune response), whereas the alternative and mannose-binding lectin pathways can be activated by C3 hydrolysis or antigens without the presence of antibodies (non-specific immune response). In all three pathways, C3-convertase cleaves and activates component C3, creating C3a and C3b, and causing a cascade of further cleavage and activation events. C3b binds to the surface of pathogens, leading to greater internalization by phagocytic cells by opsonization. C5a is an important chemotactic protein, helping recruit inflammatory cells.\(^{(35)}\) C3a is the precursor of an important cytokine (adipokine) named ASP and is usually rapidly cleaved by carboxypeptidase B. Both C3a and C5a have anaphylatoxin activity, directly triggering degranulation of mast cells as well as increasing vascular permeability and smooth muscle contraction. C5b initiates the membrane attack pathway, which results in the membrane attack complex (MAC), consisting of C5b, C6, C7, C8, and polymeric C9. MAC is the cytolytic endproduct of the complement cascade; it forms a transmembrane channel, which causes osmotic lysis of the target cell. Kupffer cells and other macrophage cell types help clear complement-coated pathogens. As part of the innate immune system, elements of the complement cascade can be found in species earlier than vertebrates; most recently in the protostome horseshoe crab species, putting the origins of the system back further than was previously thought.\(^{(33)}\)

1.2.7 Laboratory diagnosis

1.2.7.1 Light Microscopy Samples

The small, thin core of renal biopsy tissue requires special handling to prevent artifacts and, worse yet, loss, during processing. The tissue should be enclosed in lens paper or other appropriate materials developed for this purpose. This prevents loss through the cassette holes during processing. Netted bags and sponges should not be used because they almost inevitably lead to pressure-induced, mechanical artifacts. Serial sectioning at 2 to 3 μm is critical for accurate evaluation of renal biopsy material. A ribbon with 2 to 4 sections should be placed on each slide. Great care must be taken to avoid chatter, folds, or tearing. Various schemes are used involving 10 to 15 slides stained with alternating hematoxylin-eosin, periodic acid–Schiff, Jones silver, and trichrome techniques.\(^{(29)}\)

1.2.7.2 Immunofluorescence

Tissue for immunofluorescent microscopy is snap-frozen, not fixed, and sectioned in a cryostat. Cryostat sections of 2 to 4 μm thick are placed on clean, air-dried slides that are prelabeled with the name of the antigen used. The routine diagnostic kidney biopsy should be examined for the presence of immunoglobulins (IgG, IgM, and IgA), complement components (C3, C1q, C4), fibrin, and κ and λ light chains. Certain medical conditions may require more specialized studies, such as the α chains of type IV collagen in hereditary nephritis, C4d in renal transplant biopsies, among
Appropriate controls include a negative control (without antibody) and a known positive control (albumin can serve this purpose, although it has other uses as well). There are various internal positive controls, such as C3 in blood vessels, C4d in mesangial areas, IgG in protein droplets, among others. Appropriate dilution should be determined with known positive material each time a new vial of antibody is opened. A microscope fitted with a high-power epifluorescent attachment and appropriate filters is required. A skilled and experienced observer can evaluate the intensity and localization of immunoreactants while recognizing the normal background and internal positive controls for each antigen tested. Overinterpretation and underinterpretation plague the beginner and the irregular reader.\(^{(29)}\)

1.2.7.3 Immunohistochemistry

Tissue for immunohistochemistry is taken from the block also used for light microscopy. No special fixation or freezing is required. Microtome sections, cut at 2 to 3 μm, are placed on coated slides before any of several antigen retrieval steps. Certain antigen protocols require overnight processing for optimal results, whereas other techniques can be detected in 3 to 5 hours. The presence of a positive reaction can be subtle and again requires a skilled and experienced observer. Use of ×40 objective magnification or even ×100 oil objective magnification may be required to recognize certain subtle patterns in various glomerular diseases. The possible presence of such a small amount of reaction product requires excellent color titration and quality control of nonspecific background staining.\(^{(29)}\)

1.2.7.4 Transmission EM

Tissue for transmission EM is processed into plastic, then trimmed, and a 1-μm section is cut and stained, usually with toluidine blue. This section is reviewed to select an appropriate glomerulus and other structures for ultrastructural examination. These so-called thick sections may also yield diagnostic information not present on the light microscopy sections. Examples of this information include the lone atherosclerotic embolus or focal segmental glomerulosclerosis lesion. The ultramicrotome is then used to prepare the very thin sections required. The tissue is collected on a copper grid and usually stained with lead citrate and uranyl acetate.\(^{(29)}\)

1.2.7.5 Quantitative evaluation of immunofluorescence

A semi-quantitative assessment of the intensity of staining is given as mild (1+), moderate (+2) or strong (3+). Despite it's apparent subjectively result are quite reproducible between trained observers. The intensity of staining is very important on the predominance or co-dominance of IgA in glomerular deposits compared to other immunoreactants.\(^{(35)}\)

1.2.7.6 Autofluorescence

Some specimens naturally fluoresce when illuminated by light of the proper wavelength such as flavins and prophyrs, lipofuscins, elastin, collagen and formalin fixation. They complicate the use of fluorescence microscopy. Because of it's broad excitation and emission spectra. Various solutions have been proposed for the reduction or elimination of autofluorescence. One way is to chemically suppress the
autofluorescence signal with with some reagents such as 1% sodium borohydride prior staining process or toluidine blue.\(^{(36)}\)

1.2.7.7 Antigen retrieval

Antigenic determinant masked by formalin-fixation and paraffin-embedding may be exposed by enzymatic digestion. The beneficial effects of protease treatment are presumably related to cleavage of the molecular cross-links by the proteolytic enzyme, allowing the antigen to return to its normal confirmation, which serves for more effective antibody binding. A wide variety of proteases have been employed, including trypsin, proteinase K and pepsin. Concentration of the enzymes is usually 0.05 - 0.1 %, depending on type of tissue and fixation. Incubation time within 5 – 30 min. Incubation temperature is usually at 37\(^{0}\)C.\(^{(41)}\)

1.2.8 Complications of renal biopsies

Serious complications of renal biopsy are uncommon. The risk of complications will vary from centre to centre based on experience and other technical factors.\(^{(44)}\) The most common complication of kidney biopsy is bleeding. This reflects the density of blood vessels within the kidney and observation that individuals with kidney failure take longer to stop bleeding after trauma (ureamic coagulopathy). Bleeding complications include a collection of blood adjacent to or around the kidney (perinephric haematoma), bleeding into the urine with passage of blood stained urine (macroscopic haematuria) or bleeding from larger blood vessels that lie adjacent the kidney. If blood clots in the bladder, this can obstruct the bladder and lead to urinary retention.\(^{(44)}\) The majority of bleeding that occurs following renal biopsy usually resolves on its own without long-term damage. Less commonly, the bleeding may be brisk (causing shock) or persistent (causing anaemia) or both. In these circumstances, treatment with blood transfusion or surgery may be required. Surgical options to control bleeding include less invasive catheter-delivered particles to block bleeding vessels (angioembolisation) or open surgery. In most cases, bleeding can be controlled and the kidneys are not lost. Rarely, a heavily damaged kidney may need to be removed.\(^{(42)(43)}\)

Infection is rare with modern sterile operating procedures. Damage to surrounding structures, such as bowel and bladder (more likely with transplant kidney biopsy), can occur.\(^{(43)}\)

Occasionally, a biopsy will have to be abandoned prematurely due to technical issues such as inaccessible or small kidneys, obscured kidneys, difficult to penetrate kidneys or observation of bleeding complication. Further, after the biopsy has been completed, microscopic examination of the tissue may reveal heavily scarred tissue prompting recommendation for re-biopsy to avoid sampling error.\(^{(42)}\)

As with all treatments, there is a risk of allergy to the disinfectant solution, sedation, local anaesthetic and materials (latex gloves, drapes, dressings) used for the procedure.\(^{(42)(43)}\)

Finally, the biopsy needle may join an artery and vein in the kidney, resulting in the formation of an arteriovenous fistula. These usually do not cause problems and close on their own. They may be monitored over time with repeat Doppler ultrasonography. Rarely, they may result in intermittent bleeding into the urine or may
grow in size and threaten to burst. In these instances, the fistula may be closed surgically or with angioembolisation.\textsuperscript{(45)(46)}
2. Rationale and Objectives

2.1 Rationale

Accurate treatment of lupus nephritis disease depends mostly on accurate laboratory diagnosis. Immunofluorescence technique on frozen sections although are essential in this respect, is completely lacking in Sudan. Therefore introduction of such techniques on paraffin wax embedded tissue will greatly improve patients management.

2.2 General objective

To apply immunofluorescence techniques on paraffin wax embedded sections.

2.3 Specific objectives

To compare and contrast between Immunofluorescence techniques on frozen sections and paraffin wax embedded sections.
3. Material and Methods

3.1 Study area and study design

This is prospective cross sectional study done in the Renal Dialysis Center in Omdurman Military Hospital, during the period of May 2011–March 2013, on patients with Lupus Nephritis

3.2 Sample size

Comprehensive samples on all patients present in that period.

3.3 Data processing

An answer sheet was used to enter the data of the 64 patients manually and then analyzed by using SPSS (Statistical Package for Social Sciences)

3.4 Renal biopsy

Two samples were taken by experience physician from each patient by using 18-gauge needle with gentleness taking care not to stretch or crush them. One sample was placed in neutral buffered formalin container pH 7.0 for paraffin embedded sections (for 24 hours at 37°C before processing), and the other sample was placed in Optimum Cooling Temperature medium (OCT) and transferred to liquid nitrogen jar for frozen section preparation.

3.4.1 Ethical consideration

We have got a consent from all patients included in this study.

3.4.2 Preparation of renal biopsy for paraffin section.

The small thin core of renal biopsy tissue requires special handling to prevent loss during processing. The tissue was placed in lens paper. This prevents loss through the cassette holes during processing. The processing was done by using Leica automatic processing machine (see appendix) and the schedule was 20 hrs as detailed in table (3.1).
**Table 3-1 Automatic Leica processor schedule**

<table>
<thead>
<tr>
<th>No of beaker</th>
<th>Solution</th>
<th>Time</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10% Buffered formalin</td>
<td>1 hours</td>
<td>37°C</td>
</tr>
<tr>
<td>2</td>
<td>10% Buffered formalin</td>
<td>1 hours</td>
<td>37°C</td>
</tr>
<tr>
<td>3</td>
<td>50% Ethanol</td>
<td>1 hours</td>
<td>37°C</td>
</tr>
<tr>
<td>4</td>
<td>70% Ethanol</td>
<td>1 hours</td>
<td>37°C</td>
</tr>
<tr>
<td>5</td>
<td>90% Ethanol</td>
<td>1 hours</td>
<td>37°C</td>
</tr>
<tr>
<td>6</td>
<td>100% Ethanol</td>
<td>2 hours</td>
<td>37°C</td>
</tr>
<tr>
<td>7</td>
<td>100% Ethanol</td>
<td>2 hours</td>
<td>37°C</td>
</tr>
<tr>
<td>8</td>
<td>Xylene</td>
<td>1 hours</td>
<td>37°C</td>
</tr>
<tr>
<td>9</td>
<td>Xylene</td>
<td>2 hours</td>
<td>37°C</td>
</tr>
<tr>
<td>10</td>
<td>Xylene</td>
<td>2 hours</td>
<td>37°C</td>
</tr>
<tr>
<td>11</td>
<td>Paraffin wax</td>
<td>3 hours</td>
<td>37°C</td>
</tr>
<tr>
<td>12</td>
<td>Paraffin wax</td>
<td>3 hours</td>
<td>37°C</td>
</tr>
</tbody>
</table>

20 hours
3.4.2.1 Staining procedure for Formalin-Fixed Paraffin-Embedded Tissue.

1- Four micron sections were cut onto silanized slides.
2- Sections allowed to dry at 37 °C for four hours.
3- Tissue sections were dewaxed and hydrated
4- Sections were rinsed in deionized distilled water.
5- Sections were incubated in 0.1% borohydride in phosphate buffer saline for 30 minutes prior staining to remove autofluorescence due to formalin fixation.
6- Sections were washed in PBS for 10 min at 4 °C
7- Enzyme digestion was done by proteinase K for 15 min at 37°C.
8- Sections were washed in PBS for 10 minute at 4 °C
9- Sections were incubated with FITC conjugated antibodies, diluted in PBS, in a moist chamber for 20 minute at room temperature
10- Slides were washed in PBS for 10 minute at 4 °C to remove excess antiserum
11 - Slides were mounted with fluorescent mounting media.
12- Slides were examined under fluorescent microscope (see appendix).

3.4.3 Preparation of renal biopsy for frozen sections

One of the two specimens was immediately snapped-frozen in liquid nitrogen. After storage in liquid nitrogen for not longer than seven days the frozen tissue was embedded in OCT compound and sectioned 4 µm in a cryostat (see appendix) at -20°C, then placed on clean air dried slides that are pre labeled with patient number and antigen used. The sections were then stained by the direct immunofluorescence procedure.
3.4.3.1 Staining procedure for Frozen Sections

1- Four micron sections were cut by cryostat

2- Sections were fixed in acetone for 10 minute at 4 °C

3- Sections were washed in PBS for five minutes at 4 °C

4- Sections were incubated with FITC conjugated antibodies, diluted in PBS, in a moist chamber for 20 minutes at room temperature

5- Slides were rinsed in PBS to remove excess antiserum

6- Slides were washed in PBS for 10 minutes at 4 °C

7- Slides were mounted with fluorescent mounting media.

8- Slides were examined under fluorescent microscope.( see appendix)
### Table 3-2 Reagent used for Immunofluorescence staining

<table>
<thead>
<tr>
<th>Item</th>
<th>Qty</th>
<th>Unit</th>
<th>Description of article</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>Vial</td>
<td>IgG-polyclonal Ab 13 ml</td>
<td>Dako</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>vial</td>
<td>IgM polyclonal Ab 13 ml</td>
<td>Dako</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>vial</td>
<td>IgA polyclonal Ab 13 ml</td>
<td>Dako</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>vial</td>
<td>C3 polyclonal Ab 13 ml</td>
<td>Dako</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>vial</td>
<td>C1q polyclonal Ab 13 ml</td>
<td>Dako</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>vial</td>
<td>Fluorescent mounting Media 13 ml</td>
<td>Dako</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>vial</td>
<td>Phosphate buffer saline 5 liter</td>
<td>Dako</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>vial</td>
<td>Proteinase-K 100 ml</td>
<td>Dako</td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td>vial</td>
<td>Acetone 500 ml</td>
<td>Dako</td>
</tr>
</tbody>
</table>
Chapter Four

4. Results

4.1 Characteristics of the study population

Of sixty four patients, 10 were males and 54 were females (Table 4.1), of different age groups. All patient presented with lupus nephritis.

Table 4-1 Distribution of patient with lupus nephritis by sex.

<table>
<thead>
<tr>
<th></th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>10</td>
<td>15.6</td>
</tr>
<tr>
<td>Female</td>
<td>54</td>
<td>84.4</td>
</tr>
<tr>
<td>Total</td>
<td>64</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Table 4-2 The frequency of different types of lupus nephritis (n=64)

<table>
<thead>
<tr>
<th>Type of lupus</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>lupus type 2</td>
<td>2</td>
<td>3.1</td>
</tr>
<tr>
<td>lupus type 3</td>
<td>7</td>
<td>10.9</td>
</tr>
<tr>
<td>lupus type 4</td>
<td>53</td>
<td>82.8</td>
</tr>
<tr>
<td>lupus type 5</td>
<td>2</td>
<td>3.1</td>
</tr>
<tr>
<td>Total</td>
<td>64</td>
<td>100.0</td>
</tr>
</tbody>
</table>
Table 4.3 Distribution of patients with lupus nephritis by age (n=64).

<table>
<thead>
<tr>
<th>Age group</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>17 – 23</td>
<td>32</td>
<td>50</td>
</tr>
<tr>
<td>24 – 30</td>
<td>24</td>
<td>37.5</td>
</tr>
<tr>
<td>31 – 37</td>
<td>4</td>
<td>6.5</td>
</tr>
<tr>
<td>38 – 44</td>
<td>1</td>
<td>1.5</td>
</tr>
<tr>
<td>45 – 51</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>52 – 58</td>
<td>1</td>
<td>1.5</td>
</tr>
<tr>
<td>59 – 65</td>
<td>1</td>
<td>1.5</td>
</tr>
<tr>
<td>66 – 72</td>
<td>1</td>
<td>1.5</td>
</tr>
<tr>
<td>Total</td>
<td>64</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Table 4.4 Degree of positivity of IgG (Frozen and Paraffin sections)

<table>
<thead>
<tr>
<th>Result</th>
<th>GROUP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IMMUNOFLUORESCENCE IN FROZEN SECTIONS</td>
</tr>
<tr>
<td>IgG Strong Positive</td>
<td>64</td>
</tr>
<tr>
<td>Total</td>
<td>64</td>
</tr>
</tbody>
</table>
Figure 4-1 Distribution of fluorescent IgM antibody among patients with lupus nephritis (frozen and paraffin sections)
Figure 4-2 Distribution of fluorescent IgA antibody among patients with lupus nephritis (frozen sections and paraffin sections)
Figure 4-3 Distribution of fluorescent C3 antibody among patients with lupus nephritis (frozen sections and paraffin sections)
Figure 4-4 Distribution of fluorescent C1q antibody among patients with lupus nephritis (frozen sections and paraffin sections)
5. Discussion

Immunofluorescence staining of renal biopsy on frozen sections for the deposition of immunoglobulins and complement is often the primary approach for the differential diagnosis of glomerular diseases.\(^{38}\)

Previous reports of Immunofluorescence using paraffin embedded sections have included studies of lymphoid, renal, thyroid and intestinal tissue.\(^{38}(39)\) The immunofluorescent method has the advantage of higher specificity and apparently higher sensitivity over the histochemical methods.\(^{40}(41)(42)\)

The technique of direct Immunofluorescence on frozen sections to demonstrate immunoglobulins, complement, and fibrinogen is of proven value in the diagnosis of several different renal conditions, one of the major disadvantages of this procedure is the requirement for fresh frozen tissue.\(^{58}\) Problems arise because facilities for the snap-freezing of fresh specimens are not always available in all clinical areas, the transportation of frozen tissue may be difficult, especially between hospitals, and the use of special holding fixatives to allow transportation before freezing may lead to loss of antigen reactivity, the long-term storage of frozen tissue also leads to loss of antigen reactivity and finally, formalin-fixed material is sometimes the only tissue available either because the lesion was too small to divide or because stored material is being reviewed retrospectively. All of these disadvantages would be avoided if formalin-fixed paraffin-embedded tissue could be used for direct Immunofluorescence.\(^{46}\)

Qualman et al (1989), reported 80-90% rate of agreement between IF-P after trypsin digestion and IF-F with respect to the presence or absence of IgG, IgM, IgA, and fibrinogen deposition. It is possible that discrepancies between our results and others depend to some extent on the type of enzyme applied on paraffin-embedded tissues for proteolytic digestion.

Fogazzi et al (1979), found a high percentage agreement of positive and negative cases and immunofluorescence intensity for the main antigens: IgG in membranous glomerulopathy, IgA in IgA nephropathy and IgG and C1q in lupus nephritis.

The avoidance of heat retrieval was due to some previous results reported in Sudan by Ali, and Mohamadani (2005 – 2008) and other countries by Huang SN et al (1976) and Curran RC et al (1977) both reported that it is difficult to demonstrate Immunofluorescence deposits by using heat retrieval system.

In this study Immunofluorescence on paraffin sections pre-treated with proteinase-K at 37\(^\circ\)C for 15 minute revealed slightly low sensitivity than frozen section for IgM, IgA, C3, and C1, and it gives the same sensitivity for IgG, the localization and distribution patterns were similar in frozen and in paraffin sections.

C1q had the lowest Immunofluorescence sensitivity in paraffin sections when compared to frozen sections, and this difficulty may be due to insufficient incubation time to the retrieval and flourescein, while IgA, IgM and C3 showed slight differences between frozen and paraffin sections.

The advantage of IF on paraffin sections is the possibility of performing retrospective studies on older biopsies.

In summary, the present results show that it is possible to use the technique of direct Immunofluorescence to demonstrate immunoglobulins, complement, in paraffin embedded renal tissue instead of frozen sections.
6. Conclusion and Recommendation

6.1 Conclusion

Applying immunofluorescence technique on frozen sections and paraffin embedded sections reveals slight differences in sensitivity for most immunoglobulins and complement. Thus one core of renal tissue may be enough for laboratory diagnosis.

6.2 Recommendation

6.2.1 C1q was the lowest sensitive demonstrated on paraffin section by our methodology, so further study should be done to determine the optimum time for pretreatment either by enzyme digestion, or heat followed by enzyme digestion.

6.2.2 Immunofluorescence should be applied on paraffin section instead of frozen section to reduce the cost (no need for cryostat and liquid nitrogen), reduce the patient complication in renal biopsy by obtain only one biopsy to do routine stain, special stain, Immunofluorescence stain, Immunohistochemistry stain and for electron microscopy studies.

6.2.3 Studies should be continued on fluorescent technique on paraffin section by using enzyme digestion to determine the optimum method for renal biopsy.

6.2.4 Retrospective researches on achieved material should be done on other diseases such as lymphomas.
References


8. Template:Https://histo.life.illinois.edu/histo/lab/kidney/kidney.htm - "University of Illinois College of Medicine"


Appendicies

Figures:

Figure: 3-1 Leica automatic tissue processor

Figure: 3-2 Cryostat (Leica CH 1850)
The researcher examining a renal tissue by fluorescence microscope (Eurostar II)
Figure 4-5 Strong positive deposition of IgG "score III" on patient with lupus nephritis type IV (Frozen section).

Figure 4-6 Strong positive deposition of IgG "score III" on patient with lupus nephritis type IV (paraffin section)
Figure 4-7 Positive deposition of IgG "score II" on patient with lupus nephritis type IV (paraffin section)

Figure 4-8 Negative deposition of IgG on patient with lupus nephritis type IV (Frozen section)
Figure 4-9 Negative deposition of IgG on patient with lupus nephritis type IV (paraffin section)

Figure 4-10 Positive deposition of IgA "score II" on patient with lupus nephritis type IV (frozen section)
Figure 4-11 Positive deposition of IgA "score II" on patient with lupus nephritis Type IV (paraffin sections).

Figure 4-12 Positive deposition of C "score II" on patient with lupus nephritis type IV (frozen section)
Figure 4-13 Positive deposition of C "score II" on patient with lupus nephritis type IV (paraffin section)