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Exploring the Role of Immunofluorescence in Renal Biopsies: A Study on Nephrotic Syndrome and Glomerulonephritis

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Abstract

Immunofluorescence (IF) is an assay for detecting and visualizing the presence of specific proteins in tissues. IF is often used as a supporting examination to help in the diagnosis of renal disease, especially in nephrotic syndrome, and glomerulonephritis. This study aims to determine the role of IF in detecting and visualizing the presence of proteins, such as antigens, immune complexes, antibodies circulating, and complements in the renal tissues. This study uses renal tissue specimens from patients with renal disease that have been treated according to the immunofluorescence protocol at the Department of Anatomical Pathology. The antibodies used include human anti-IgM, human anti-IgG, combination of human IgG-IgM-IgA antibodies (polyvalent antibodies), and human anti-C3 antibodies. The renal tissue specimens treated with immunofluorescence protocol were examined using a light microscope that has a fluorescence filter to obtain the glomerular structure. Immunofluorescence using human anti-IgM antibodies showed a granular pattern and mild positivity. Immunofluorescence using human anti-IgG antibodies showed a smudge pattern and weak positivity. Immunofluorescence using human anti-C3 antibody showed moderate positive staining. Moreover, immunofluorescence using polyvalent antibodies on renal tissue from nephrotic syndrome patients showed strong and diffuse positive staining. Immunofluorescence of renal tissue specimens with several antibodies showed a difference in pattern and positivity. Therefore, IF can be used as a supporting examination to help in determining the diagnosis of several renal diseases in the patient, because IF can detect and visualize the presence of proteins in the renal tissues.

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Introduction

Immunofluorescence (IF) is an immunochemistry technique used to detect antigens in various cells and tissues. The basic principle of IF is antigen detection by antibody-tagged fluorophores. The signal emission of fluorescence is a positive sign of the presence of an antigen-antibody bond. Based on that principle, IF is highly sensitive compared with Immunohistochemistry [1]. Beside that, IF has many advantages such as signal amplification, high specificity, sensitivity, resolution, and analytical capability. However, IF needs a fluorescence microscope to observe fluorescence signals and to read immunological-specific reactions in the cells and tissues [2]. So, IF has limitations compared with Immunohistochemistry (IHC) which can be observed with a light microscope.

There are four major types of IF techniques. First, direct IF is a one-step detection of cell and tissue antigens with an antibody labeled fluorophore. Second, indirect IF is two-step antigen detection which uses primary free-label antibody bound to antigen molecule and secondary labeled fluorophore antibody bound to primary antibody. Third, Indirect Immunofluorescence Complement-Fixation (IIF-CF) is the binding of antigen and antibody to generate C3 complement activation. This technique gives more signal amplification than other types of IFS. Fourth, double IF is the type of IF that can detect two or more antigens with two or more tagged antibodies. In this method, each antibody has a different fluorophore to distinguish antigens (ie, FITC and Rhodamine) [2].

IF has been widely used as a diagnostic tool for viral infection [3], autoimmune disease [2], and renal disease[4] (i.e., nephrotic syndrome). Nephrotic syndrome occurs in children, with a prevalence of 16 cases per 100,000 children. Idiopathic nephrotic syndrome is known for its Minimal Change Disease (MCD), diffuse Mesangial Hypercellularity (MH), and Focal Segmental Glomerulosclerosis (FSGS) [5]. Histological patterns of renal disease are challenging to diagnose with a light microscope, such as Early membranous glomerulonephritis, IgA nephropathy, and FSGS are challenging to observe with light microscopy. Thus, IF becomes a secondary diagnostic test. IgM nephropathy was first discovered in 1978 as glomerulonephritis [6].

Several antibodies that have been used as standard diagnostic tests to differentiate between nephrotic and nephritic syndrome are antibodies against IgA, IgM, IgG, C3, C4, and C1q. Immunofluorescence analyzing renal biopsies remains part of the diagnostic procedure. Combination of diagnosis with light microscopy and IF detected more nephrotic syndrome cases followed by IgM, MCD, FSGS, Chronic sclerosing glomerulonephritis, Mesangial Proliferative Glomerulonephritis (MPGN), Mesangial Capillary Hlomerulonephritis (MCGN), IgA nephropathy Crescentic glomerulonephritis, lupus nephritis, and others [7]. IgM+ IF can be used as a marker of disease severity in children with nephrotic syndrome with MCD, MH, and FSGS [5]. Adult-onset nephrotic syndrome was evaluated clinically by direct method using antibodies against IgG, IgM, IgA, and C3 [6]. In this study, we used IF as a diagnostic tool for nephrotic syndrome and nephritic syndrome. The antibody used are IgM, IgG, IgA, C1q, and C3. The signal emission is read by a fluorescence microscope. This study aims to determine the role of IF in detecting and visualizing the presence of proteins, such as antigens, immune complexes, antibodies circulating, and complements in the renal tissues.

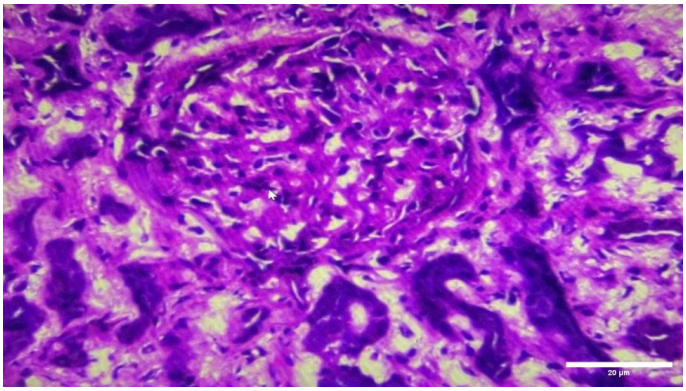

Figure 1: Glomerular structure observed under light microscope (400x magnification and 20µm scale bar) before using fluorescence filter.

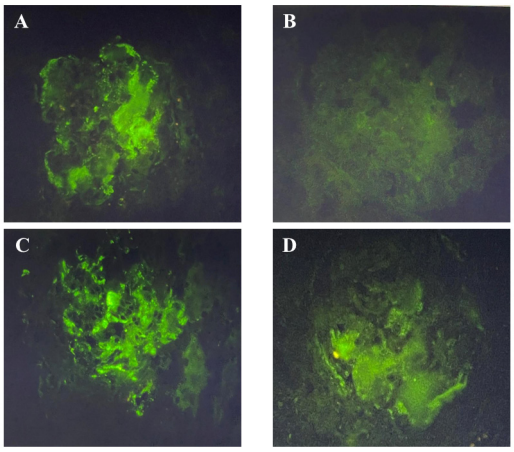

Figure 1: Immunofluorescence results on glomerulus. A. Human anti-IgM antibodies (mild IgM deposition), the localization and pattern (granular, linear, or diffuse) provide insight into the underlying condition. B. Human anti-IgG antibodies (weak IgG deposition). C. Human anti-C3 complement antibody (moderate C3 deposition), suggesting a noticeable but not excessive deposition of the C3 complement. D. Human polyvalent antibody (strong polyvalent deposition) on renal tissue with nephrotic syndrome, this result points to significant immune-mediated injury in the renal, often involving the glomeruli.

Methods

The experimental session was conducted on Wednesday, November 20, 2024, at the Anatomical Pathology Laboratory, Department of Anatomical Pathology, Faculty of Medicine, Universitas Indonesia, Jakarta. This experimental session uses renal tissue specimens, specifically glomerular tissue from nephrotic syndrome patients, which have been treated according to the immunofluorescence protocol at the Department of Anatomical Pathology. The antibodies used in this study include monovalent antibodies, polyvalent antibodies, and antibodies against opsonin proteins. Monovalent antibodies use human anti-IgM and human anti-IgG antibodies. Polyclonal antibodies use a combination of human anti-IgM, human anti-IgG, and human anti-IgA antibodies. Meanwhile, antibodies against opsonin proteins use human anti-C3 antibodies.

Result

Sample slides were examined using a light microscope to obtain the glomerular structure that was going to be observed (Figure 1). Using a fluorescence filter, immunofluorescence of sample slides was observed (Figure 2). Immunofluorescence using IgM antibodies was observed with a granular pattern and

mild positivity (Figure 2A). Some fluorescence was diffused evenly with a smudge pattern, which usually shows an uneven accumulation of deposits along the Glomerular Basement Membrane (GBM). The localization and pattern (granular, linear, or diffuse) provide insight into the underlying condition. Immunofluorescence using IgG antibodies was observed in a smudge pattern and weak positivity (Figure 2B). Another immunofluorescence using C3 complement antibody gave (mild) staining, which indicates moderate positive staining, suggesting a noticeable but not excessive deposition of the C3 complement (Figure 2C). Immunofluorescence using polyvalent antibodies, which are a combination of IgM, IgG, and IgA, with (strong) staining showed a very strong immunoglobulin deposition in the glomerular (Figure 2D). This might suggest either extensive immune complex deposition, a severe immune-mediated disease, chronic inflammation, or an intense ongoing immune response.

Discussion

The immunofluorescence results on the glomerular tissue of a nephrotic syndrome patient reveal the presence of varying deposits of immunoglobulins and complements, depending on the type of antibody used. Strong fluorescence with polyvalent antibodies (human anti-IgM, human anti-IgG, and human anti-IgA antibodies) indicates a significant accumulation of immune complexes in the glomerulus. Positivity with monovalent antibodies such as human anti-IgM, human anti-IgG, and human anti-complement antibodies like C1q and C3 confirms the activation of the classical and alternative complement pathways. This activation is mediated by the binding of immune complexes to C1q, which triggers the complement cascade leading to the formation of C3 convertase, resulting in localized inflammation and cellular damage.

Patho mechanistically, the nephrotic syndrome often involves the deposition of immune complexes formed from antigens and antibodies in the glomerular basement membrane [8]. These immune complexes activate the complement system, causing the release of inflammatory mediators, such as anaphylatoxins (C3a, C5a), which recruit leukocytes to the site. The accumulation of leukocytes and the release of proteolytic enzymes and free radicals cause damage to podocytes and the glomerular basement membrane. This damage disrupts the selective permeability of the glomerulus, leading to massive proteinuria, a hallmark of nephrotic syndrome. Complement deposits, particularly C1q, suggest the involvement of the classical pathway, which is often associated with autoimmune diseases like lupus nephritis. Additionally, the high intensity of fluorescence observed with polyvalent antibodies (human anti-IgM, human anti-IgG, human anti-IgA antibodies) may indicate membranous glomerulonephritis or other conditions involving complex immune mechanisms. These findings underscore the critical role of the immune response in the pathogenesis of nephrotic syndrome and highlight that controlling inflammation and managing immune complex deposits are key strategies in its treatment [9,10].

The differential diagnosis in glomerular immunofluorescence examination includes IgA nephropathy (IgAN), acute postinfectious glomerulonephritis, membranous nephropathy, C3 glomerulopathy, and lupus nephritis.[11] Variations in fluorescence patterns observed in the glomerulus can help distinguish between these diseases. These differences arise from distinct mechanisms of glomerular injury that occur in each disease. There are three primary mechanisms of glomerular injury: (1) deposition of circulating immune complexes in the subendothelial or subepithelial regions of the glomerulus; (2) in situ immune complex formation in the glomerulus due to the binding of circulating antibodies to antigens present in the glomerulus; and (3) formation of immune complexes on the glomerular basement membrane (GBM) by anti-GBM antibodies. Glomerular injury involving mechanisms 1 and 2 typically results in a granular fluorescence pattern, whereas mechanism 3, involving anti-GBM antibodies, produces a linear fluorescence pattern [11,12].

IgAN involves the deposition of immune complexes containing IgA, resulting in a positive granular fluorescence pattern for IgA, with or without positive staining for IgG, IgM, and C3 in the renal mesangium [11,12]. More dominant C3 involvement is often observed in IgA-dominant infection-associated glomerulonephritis, commonly occurring after Staphylococcus infections. This characteristic can help distinguish IgAN from IgA-dominant infection-associated glomerulonephritis [13,14]. The C3 fluorescence pattern in IgA-dominant infection-associated glomerulonephritis may display a starry-sky pattern due to the involvement of capillary walls and the mesangium in the glomerulus [14].

Acute postinfectious glomerulonephritis can result from the deposition of immune complexes or in situ immune complex formation in the glomerulus, which produces a granular fluorescence pattern for IgG and C3 distributed across the GBM and mesangium [12]. Membranous nephropathy is a renal disease caused by the formation of anti-podocyte antibodies, which deposit as immune complexes between the GBM and podocytes, manifesting as nephrotic syndrome [15]. The immunofluorescence pattern in membranous nephropathy shows diffuse granular staining for IgG and C3 along the GBM [11]. Although rare, positive immunofluorescence staining for IgM, IgA, or C1q can also occur in membranous nephropathy with evenly distributed patterns [12].

C3 glomerulopathy can manifest as either nephrotic or nephritic syndrome, with a dominant C3 fluorescence pattern showing either granular or semi linear staining across the intramembranous, mesangial, and capillary wall regions. C3 positivity in C3 glomerulopathy is typically two-fold higher than other immunoglobulins or C1q [16]. In lupus nephritis, immunofluorescence reveals a “full house” pattern characterized by granular staining for all three classes of immunoglobulins—IgA, IgM, and IgG—along with C3 and C1q, primarily distributed in the subendothelial region [17].

The immunofluorescence pattern observed in the glomerulus does not always indicate glomerular damage or pathology. Fluorescence can also be present under normal conditions and is often used as an internal control in renal immunofluorescence examinations.

False-negative results in renal immunofluorescence can impact both diagnosis and treatment decisions. Several factors can contribute to this condition, including the use of fresh tissue instead of Formalin-Fixed, Paraffin-Embedded (FFPE) tissue. FFPE processing can cause masking of immune complexes, leading to false-negative results. This has been demonstrated by the lower sensitivity of immunofluorescence when using FFPE tissue compared to fresh tissue.[18] Additionally, pre-analytical factors, such as the sample fixation process, and analytical steps, including suboptimal enzymatic treatments during immunofluorescence staining, may also result in false-negative outcomes in renal immunofluorescence examination [19].

Conclusion

This study showed the ability of IF to reveal distinct fluorescence patterns and positivity, aiding in the differentiation of renal diseases. The strong and diffuse positivity of the fluorescence indicates extensive immune complex deposition that causes a significant immune-mediated injury in the renal. While mild or weak positivity indicates the early stage or mild disease activity of the renal disease. Furthermore, the diagnostic precision of renal disease can be obtained and guide the clinicians to make the best therapeutic strategies.

Author Statements

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