

**Detection of Laminin beta-1 Protein Localization in Lupus Nephritis Using Immuno-gold  
Electron Microscopic Technique**  
**การตรวจหาการแสดงออกของโปรตีน Laminin beta-1 ในผู้ป่วย Lupus nephritis (LN) ด้วยเทคนิค  
Immuno-gold electron microscope**

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**ABSTRACT**

Kidney disease Lupus nephritis (LN) is a disease that occurs from the body's immune system, destroys itself in the kidney area. The cause of the disease comes from allergies. SLE causes significant renal symptoms of nephritis, including swelling, bubbles. Detecting protein in urine high blood pressure whole swelling, acute renal failure (due to severe nephritis) and chronic LN syndrome have a high cumulative incidence in Asian (55%), African (51%) and Spanish (43%) compared to White people (14%) (1) to 25% of these patients continue to develop end-stage renal disease (ESRD) 10 years after the onset of renal dysfunction in terms of diagnosis results over a period of time. 5 and 10 years, the survival rate of kidney LN in 1990 is between 83-93% and 74-84% respectively (C.C. Mok. 2010) depends on organs that are abnormal Causes of death include renal failure due to studies of lupus nephritis or allergies in various classes of 3-6 classes. The results from nine cases, the samples studied were aged 14-52 years were all women of lupus nephritis, PA02, PA03, PA04, PA05, PA06, PA07, PA08, PA09, PA10, PA11 and one case control (PA01) in Srinagarind hospital, Khon kaen, Thailand consists of Class III, IV and V by the Immuno-gold electron microscopic technique. Laminin beta-1 was found in all classes but the difference was found. The most common class was Class IV, the lowest class III combined with Class V in position glomerular basement membrane (GBM) and Mesangial matrix. In conclusion, our findings are compatible with previous studies that nucleosomes derived from apoptotic cells are trapped within glomerular basement membranes during the development of lupus nephritis. Here, circulating anti-dsDNA antibodies may cause nephritis through binding the planted antigens in situ, although the possibility that the nucleosomes also could bind GBMs as part of preformed nephritogenic immune-complexes cannot be excluded and conclude that aberrant laminin beta-1 is an intrinsic ligand of the GBM, in feature could be of a significant pathobiologic interest during the development of lupus nephritis. The results indicate that in Thai people, there is a mechanism similar to that in the previous reports. In addition, we also find that there is also aberrant expression of laminin beta-1 in Class III and Class V, and give us a further understanding that the mechanism of LN can be planted antigen immune complex formation, in addition to circulating immune complex formation. Which is good for treatment of allergies The mechanism of birth as well as information on the production of anti-allergic anti-protein drugs can be cured in the future.

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### บทคัดย่อ

โรคไต Lupus nephritis (LN) เป็นโรคที่เกิดขึ้นจากระบบภูมิคุ้มกันของร่างกายทำลายตัวเองที่บริเวณไตสาเหตุของโรคมาจากโรคภูมิแพ้ SLE ทำให้เกิดอาการทางไตที่สำคัญของไตอักเสบ ได้แก่ บวม ปัสสาวะเป็นฟอง ตรวจพบโปรตีนในปัสสาวะ ความดันโลหิตสูง อาการบวมทั้งตัวไตวายทั้งแบบเฉียบพลัน(เนื่องจากไตอักเสบรุนแรง)และแบบเรื้อรัง โรคLN มีอุบัติการณ์สะสมสูงในคนไทย (55%), แอฟริกัน (51%) และสเปน (43%) เมื่อเทียบกับชาวผิวขาว (14%) (1) ถึง 25% ของผู้ป่วยเหล่านี้ยังคงพัฒนาระยะสุดท้ายโรคไตวายเรื้อรัง (ESRD) 10 ปีหลังจากที่เริ่มมีอาการผิดปกติของไตในแง่ของผลการวินิจฉัย ในระยะเวลา 5 และ 10 ปีอัตราการอยู่รอดของไต LN ในปี 1990 อยู่ระหว่าง 83-93% และ 74-84% ตามลำดับ (C.C. Mok, 2010) ขึ้นกับอวัยวะที่มีความผิดปกติ สาเหตุของการเสียชีวิตได้แก่ ภาวะไตวาย จากผลการศึกษาโรคไตอักเสบลูปัสหรือโรคภูมิแพ้ในคลาสต่างๆ 3-6 คลาสของผู้ป่วย 9 รายพบว่า ช่วงอายุอยู่ระหว่าง 14-52 ปี ซึ่งเป็นผู้หญิงทั้งหมด ในโรงพยาบาลศรีนครินทร์ จังหวัดขอนแก่น ในประเทศไทย ประกอบด้วย Class III, IV and V โดยใช้วิธี Immuno-gold electron microscopic technique ปรากฏว่าพบ Laminin beta-1 ในทุก Class ที่ศึกษาแต่ปริมาณที่พบมีความแตกต่างกัน Class ที่พบมากที่สุดคือ Class IV ส่วนคลาสที่พบน้อยที่สุดคือ Class III combined with Class V ตำแหน่งที่พบโปรตีน ลามินินเบต้าวันคือ Glomerular basement membrane (GBM) และที่ Mesangial matrix จากผลการทดลอง โรค lupus nephritis มีการแสดงออกของ Laminin ที่ผิดปกติ โดยทำให้โครงสร้างโปรตีนที่ GBM เป็นชนิดที่มีองค์ประกอบเป็น beta-1 ซึ่งเคยพบว่าเป็นที่เกาะของ nucleosomes ซึ่งเมื่อจับกับ antibodies จะกระตุ้นให้เกิด immune reaction และทำให้เกิด Lupus nephritis ตามมา ซึ่งการค้นพบสอดคล้องกับที่เคยมีในรายงาน proliferative Lupus nephritis (LN) มาก่อนแต่ยังไม่พบว่ามีการรายงานใน Class อื่นๆ จากทดลองทำให้ทราบว่าคนไทยมีกลไกการเกิดโรคที่คล้ายคลึงกับในรายงานก่อนหน้านี้ของต่างประเทศแต่ยังไม่มีการศึกษาในประเทศไทยรวมทั้งพบการเปลี่ยนแปลงใน Class III combines with Class V อีกด้วย และทำให้เข้าใจมากขึ้นว่ากลไกการเกิด LN มีกลไกอื่นร่วมด้วยคือ Planted antigen immune complex formation นอกเหนือจากการที่มี circulating immune complex formation ซึ่งจะเป็นผลดีในการรักษาโรคภูมิแพ้ กลไกการเกิดรวมทั้งเป็นข้อมูลในการผลิตยาต้านโปรตีนรักษาโรคภูมิแพ้ให้หายขาดได้ในอนาคต

**Keywords:** Lupus nephritis Laminin beta-1 Glomerular basement membrane (GBM)

**คำสำคัญ:** โรคไตอักเสบลูปัส โปรตีนลามินิน เบต้าวัน ตำแหน่งของไต GBM

### Introduction

Kidney disease Lupus nephritis (LN) is a disease that occurs from the body's immune system, destroys itself in the kidney area. The cause of the disease comes from allergies. SLE causes significant renal symptoms of nephritis, including swelling, bubbles. Detecting protein in urine high blood pressure whole swelling, acute renal failure (due to severe nephritis) and chronic. The overall prevalence and incidence of lupus nephritis (LN) in the range of number 1.4-21.9% and 7.4-159.4 cases per 100,000 patients respectively (Ortiga et al., 2010). The first allergy study in Thailand in 1995 (Shayakul et al.1995) According to the kidney disease register of the kidney association of Thailand, in 2014 Lupus nephritis was the 6<sup>th</sup> leading cause of the chronic kidney disease (Nephrology society of Thailand, 2014) LN is clinically evident in 50-60% of patients with systemic lupus erythematosus (SLE), and it is histologically evident in most SLE patients, even those without clinical manifestations of renal disease. Evaluating renal function in SLE patients

is important because early detection and treatment of renal involvement can significantly improve renal outcome. LN is an immune complex-mediated glomerular disease. It has recently become clear that the nucleosome has a particular pathogenic impact on lupus nephritis. Nucleosome are proven major autoantigen that drive T-cell-dependent autoimmune responses (Mohan, 1993). It has been suggested glomerular basement membrane (GBM) itself does not bind lupus autoantibodies in vivo. Planted nucleosomes on GBM and mesangial matrix has been proposed as an initial step in the pathogenesis of LN. These nucleosomes may originate from circulating or intra-glomerular apoptotic cells, resulting in both cases in the formation of sub-endothelial and sub-epithelial deposits. The nephritogenic autoantibodies could bind to GBM-associated nucleosomes if the nucleosomes are not already trapped in the immune complexes with anti-double stranded DNA (anti-dsDNA) antibodies in the circulation (Kalaaji et al., 2006).

The laminins represent a family of large glycosylated proteins largely confined to basement membranes. In the kidney, laminin 11 is the apparent constituent of fully mature normal GBM, replacing laminin 1 during development (Miner, 1999). The kidney mesangial matrix contains laminin 1, laminin 2, laminin 8, and laminin 10, while in tubular basement membranes contain laminin 1 (proximal tubule), laminin 5 (collection duct), and laminin 10 (distal tubule and collection duct). Laminin beta-1 is a peptide chain that binds to the laminin receptor. Laminins, a family of extracellular matrix glycoproteins, are the major non collagenous constituent of basement membranes. They have been implicated in a wide variety of biological processes including cell adhesion, differentiation, migration, signaling, neurite outgrowth and metastasis. Laminins are composed of 3 non identical chains: laminin alpha, beta and gamma (formerly A, B1, and B2, respectively) The laminin beta 1 chain has 7 structurally distinct domains, which it shares with other beta chain isomers. Laminin, beta 1 is expressed in most tissues that produce basement membranes, and is one of the 3 chains constituting laminin 1, the first laminin isolated from Engelbreth-Holm-Swarm (EHS) tumor. A sequence in the beta 1 chain that is involved in cell attachment, chemotaxis, and binding to the laminin receptor was identified and shown to have the capacity to inhibit metastasis. (Ikonen et al., 1989).

### **Objective of the study**

Study on Laminin beta-1 localization in various classes of LN using Immuno-gold electron microscope technique.

### **Materials and methods**

Nine LN patients with kidney biopsy proven are included in the study. The kidney biopsy was immersed in 4% Para formaldehyde solution to prevent protein degradation. After that, the kidney is divided into two parts, the first part being diagnosed by pathology. The second part is studied by Immuno-gold electron microscope and is diagnosed by pathologist. There were nine LN patients diagnosed with LN and two non-LN patients at Srinagarind Hospital. Formula of sample size,  $n = \text{sample size}$ ,  $Z = 1.960$ ,  $e = 0.05$   $\sigma = \text{Population} / \text{Past surveys}$ . (76 from incidence of lupus nephritis in the range of 1.4-21.9% and 7.4-159.4 cases per 100,000 patients respectively  $\pm 7\%$  value error. (Ortiga et al., 2010).

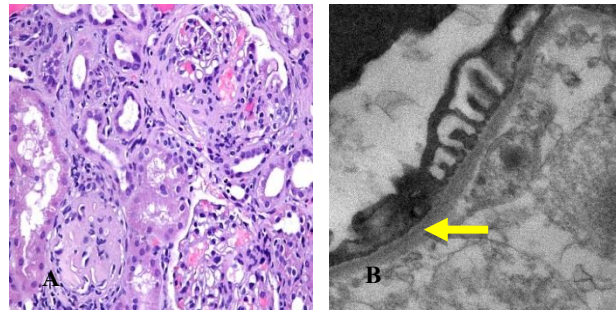
**Immuno-gold and EM technique** The kidney biopsy contents of nine patients diagnosed are LN from pathologist of Srinagarind hospital. Khon Kaen, Thailand. The kidney biopsy contents of two patients diagnosed are not LN from pathologist of Srinagarind hospital. Khon Kaen, Thailand. Basic data collection such as sex, age, ethnicity and patient's history were used. Data were collected from the database of Srinagarind hospital. Transmission biopsy size 1x0.4x0.4 cm LN patients were placed in a test tube containing 5 ml Michel preserve tissue solution and EM solution at 4°C and then divided into 2 parts: Part 1 size 0.5x0.4x0.4 cm part 1x0.4 x0.4 cm. Part 2 size 0.5x0.4x0.4 cm, respectively. Pieces of kidney biopsy, part 1 into study pathological processes such as H & E and Immuno-fluorescent techniques. Pieces of kidney biopsy, part 2 fixed 4% paraformaldehyde into the EM method. EM method contents: the first step kidney biopsy (part 2 for EM) fix 4% paraformaldehyde in phosphate buffer at 4°C for at least 12 hours. Then frozen sections 15 nm. And fix in 4% paraformaldehyde, wash with PBS and blocking with blocking solution 30 min/BSA. Add Primary Laminin beta1 with 0.1% BSA/0.002% saponin/BSA Overnight 4°C and wash with 0.002 % saponin/PBS 10 min x 3 times. Add Secondary Antibody-gold reaction 1/100 dilute with 0.1% BSA/0.002 % saponin/PBS 3-4 hrs. at RT and wash with 0.002% saponin/PBS 10 min x 3 times. Add Silver enhancement 90 min at RT and wash with PB 10 min x 3 times. Fix tissue in 1% Os/PB 30 min and wash distil water 3 time 5 min. Dehydration in various alcohol, 70 % alcohol 1 time 5 min, 80 % alcohol 1 time 5 min, 90 % alcohol 1 time 5 min, 95 % alcohol 1 time 5 min, 100 % alcohol 3 times 20 min. Add pure Epon, RT overnight and discard Epon, Replace with New Epon, 60 °C, 48 Hr. Keep in desiccator until the next step in room temp. Then section Block (Epon stub) with ultrathin section under ultra microtome 70 nm. Stain with Uranyl acetate & Lead citrate and dry up at room temp. Observe under TEM and Photograph and show flow EM method.

## Results

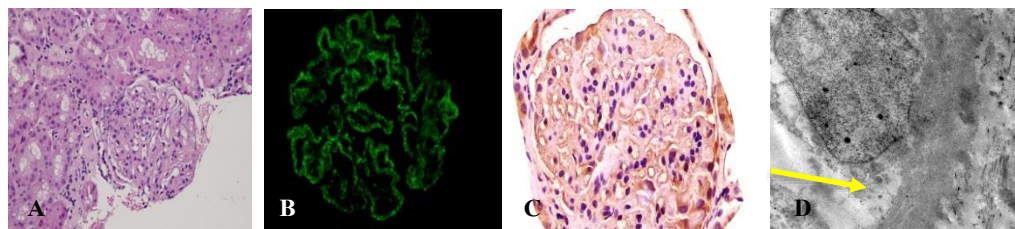
From the results of the experiment patients of Srinagarind hospital, Khon Kaen, Thailand. Using the immunoglobulin-Gold electron microscopic technique, it shows the stickiness of gold particles. According to various images with arrows pointing at the GBM, Mesangial, and Matrix position. In table 1 Show various class of Lupus nephritis and result EM localization. Results from nine case, The sample study are age 14-52 years these all women of lupus nephritis, PA02, PA03, PA04, PA05, PA06, PA07, PA08, PA09, PA10, PA11 and one case control(PA01) consists of Class III, IV and V by the Immuno-gold electron microscopic technique.

Laminin beta-1 was found in all classes but the difference was found. The most common class was Class IV, the lowest class III combined with Class V in position Glomerular basement membrane (GBM) and Mesangial matrix.

Findings are compatible with previous studies that nucleosomes derived from apoptotic cells are trapped within glomerular basement membranes during the development of lupus nephritis. Here, circulating anti-dsDNA antibodies may cause nephritis through binding the planted antigens in situ, although the possibility that the nucleosomes also could bind GBMs as part of preformed nephritogenic immune-complexes cannot be excluded and conclude that aberrant laminin beta-1 is an intrinsic ligand of the GBM, in feature could be of a significant pathobiologic interest during the development of lupus nephritis. In each class There are differences as shown in the figure below.

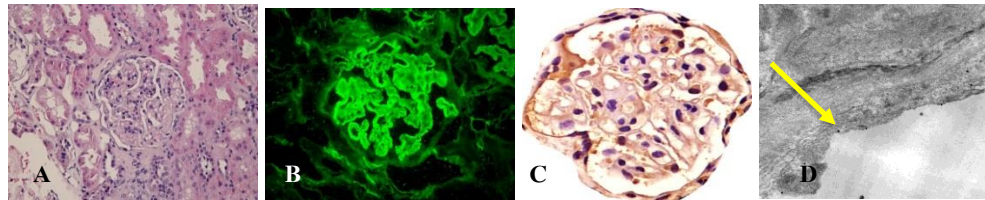


**Figure 1** (From case No.PA01) Control: A: Glomeruli exhibit moderate hypercellularity of the mesangial cells and mild increase in mesangial matrix. (x40). B: Ultrastructure of glomerular basement membrane (GBM) in IgA nephropathy show negative staining (Magnifications x5,000). Showing glomerular basement membrane (GBM) (arrow). (x17,000)

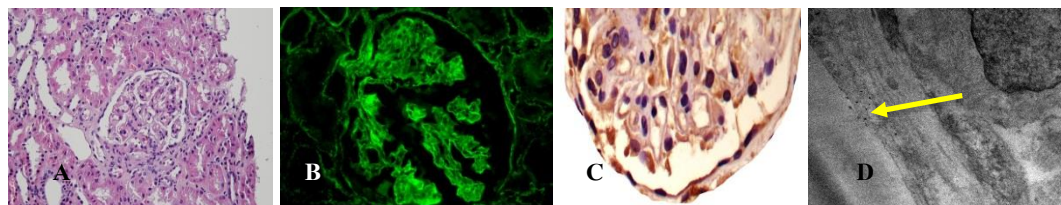


**Figure 2** (From case No.PA02) Lupus nephritis class III (A/C) combined with Class V. A: Glomerular structure staining with H&E LN class III (A/C) combined with Class V. (x40) The glomeruli reveal mild enlargement with global hypercellularity due to mild increase in mesangial cells and mild increase in mesangial matrix. B: Immunofluorescence finding. (x40) Granular staining of the peripheral capillary for IgG (2+), IgM (1+), IgA (1+), C3 (2+), C4 (1+), C1q (2+), kappa (3+), lambda (3+) and fibrin (trace) are noted. C: Glomerulus stain with laminin beta-1 antibody by immunohistochemistry (IHC) technique. (x40) GBM have positive staining, which is dyed with laminin beta-1 at GBM. D: Electron microscopic finding. Using laminin beta-1 antibody and the protein a-gold complex. The gold particles are found in lamina rara externa of glomerular basement membrane. (arrow) (x17,000)

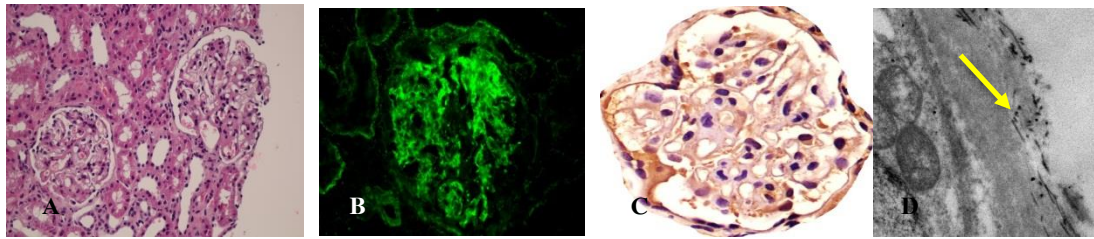




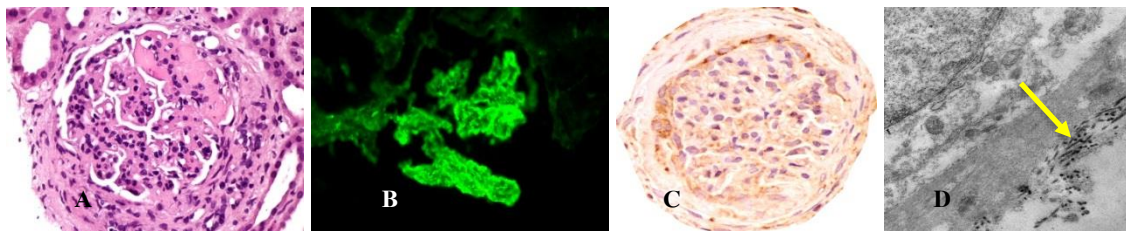
**Figure 3** (From case No.PA03) Lupus nephritis class III+V. A: Glomerular structure staining with H&E, LN class III + V. (x40) glomeruli exhibit mild hypercellularity of the mesangial cells and mild increase in mesangial matrix. B: Immunofluorescence finding. (x40) glomeruli show granular mesangial deposits of IgG (3+), IgM, (1+), IgA(1+), C3(1+), 4(1+), C1q(3+), kappa(3+), lambda(3+) and fibrin (1+). C: Glomerulus stains with laminin beta-1 antibody by immunohistochemistry (IHC) technique. (x40) At locate GBM have brown staining, which is dyed with lamini beta-1. D: Electron microscopic finding. Using laminin beta-1 antibody and the protein a-gold complex. The gold particle are found in layers lamina rara interna between lamina densa of glomerular basement membrane. (arrow) (x17,000)



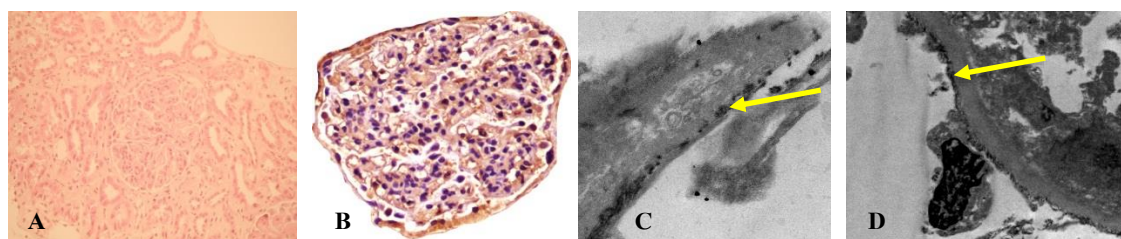
**Figure 4** (From case No.PA04) Lupus nephritis class IV-G (A) with regression to class II. A: Glomerular structure staining with H&E, LN class IV-G (A). (x40) Most of the glomeruli exhibit mildly increased hypercellularity of the mesangial cells and increase in mesangial matrix. B: Immunofluorescence finding. (x40) glomeruli show capillary wall and mesangial deposits of IgG(3+), IgM,(2+), IgA(2+), C3(2+), C4(1+), C1q(3+), kappa(2+), lambda(3+) and fibrin (+/-). C: Glomerulus stain with laminin beta-1 antibody by immunohistochemistry (IHC) technique. (x40) GBM have positive staining, which is dyed with laminin beta-1. D: Electron microscopic finding. Using laminin beta-1 antibody and the protein a-gold complex. The gold particle is found in layers lamina rara interna of glomerular basement membrane. (arrow) (x17,000)



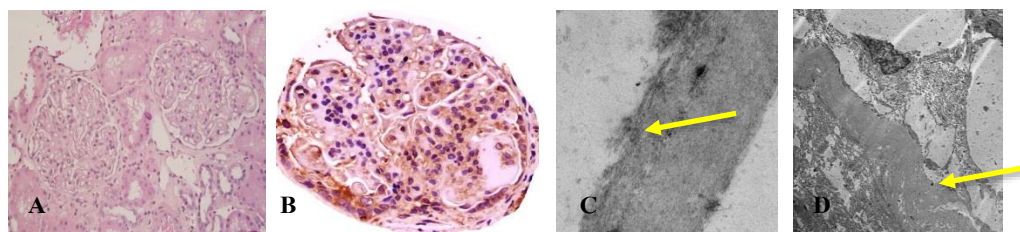
**Figure 5** (From case No.PA05) Lupus nephritis class IV-G (A). A: Glomerular structure staining with H&E, LN class IV-G (A). (x40) Glomeruli reveal global hypercellularity with predominant mesangial and focal segmental endocapillary proliferation. B: Immunofluorescence finding. (x40) Glomeruli are received. Granular capillary wall and mesangial staining of IgG (2+), IgM (1+), IgA (2+), C3 (3+), C4 (trace), C1q (2+), fibrinogen (2+), Kappa (3+), and Lambda (2+) are present. C: Glomerulus stain with laminin beta-1 antibody by immunohistochemistry (IHC) technique. (x40) GBM have positive staining, which is dyed with laminin beta-1. D: Electron microscopic finding. Using laminin beta-1 antibody and the protein a-gold complex. The gold particle is found in layers lamina rara interna of glomerular basement membrane. (arrow) (x17,000)



**Figure 6** (From case No.PA07) Lupus nephritis class IV-G (A/C). A: Glomerular structure staining with H&E, LN class IV-G (A/C). (x40) Almost of the glomeruli reveal moderate enlargement with global hypercellularity due to marked increase in mesangial cells and mild increase in mesangial matrix. B: Immunofluorescence finding. (x40) Glomeruli show diffuse, fine to granular staining of the peripheral capillary wall and mesangium for IgG (3+), IgM (3+), IgA (2+), C3 (3+), C4 (2+), C1q (3+), kappa (2+), lambda(2+) and fibrin (2+) are noted. C: Glomerulus stain with laminin beta-1 antibody by immunohistochemistry (IHC) technique. (x40) GBM have positive staining, which is dyed with laminin beta-1. D: Electron microscopic finding. Using laminin beta-1 antibody and the protein a-gold complex. The gold particle is found in layers lamina rara interna of glomerular basement membrane. (arrow) (x17,000)



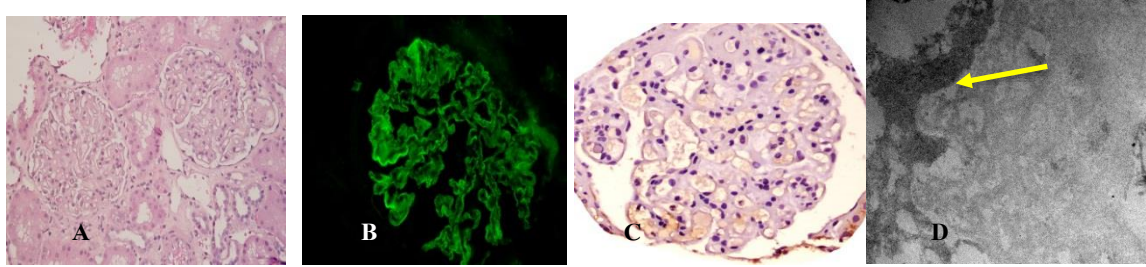
**Figure 7** (From case No.PA08) Lupus nephritis class IV-G (A/C). A: Glomerular structure staining with H&E, LN class IV-G (A/C). (x40) All of the glomeruli reveal enlargement with global hypercellularity and prominent lobular architecture. Moderate increase in mesangial cells and mesangial matrix (giving a nodular appearance) are noted. B: Immunofluorescence finding. (x40) Glomeruli shows granular staining of the peripheral capillary wall and minimal mesangium for IgG (3+), IgM(1+), IgA (2+), C3 (3+), C4 (2+), C1q (3+), fibrin (1+), kappa (2+), and lambda (2+). C: Glomerulus stain with laminin beta-1 antibody by immunohistochemistry (IHC) technique. (x40) GBM have positive staining, which is dyed with laminin beta-1. D: Electron microscopic finding. Using laminin beta-1 antibody and the protein a-gold complex. The gold particle is found in layers lamina rara externa of glomerular basement membrane. (arrow) (x17,000)



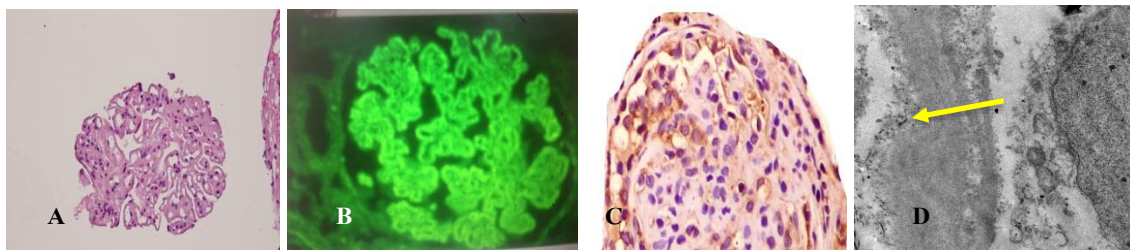
**Figure 8** (From show case No.PA09) Lupus nephritis in class IV-G (A) plus class V.

A: Glomerular structure staining with H&E, LN class IV-G (A) plus class V. (x40) All of the glomeruli reveal enlargement with global hypercellularity and prominent lobular architecture. Moderate increase in mesangial cells and mesangial matrix. B: Immunofluorescence finding. (x40) Glomeruli shows granular staining of the peripheral capillary wall staining for IgG (3+), IgM (1+), IgA (2+), C3(2+), C4(2+), C1q (3+), fibrin (2+), kappa (3+), and lambda (3+). C: Glomerulus stain with laminin beta-1 antibody by immunohistochemistry (IHC) technique. (x40) GBM have positive staining, which is dyed with laminin beta-1. D: Electron microscopic finding. Using laminin beta-1 antibody and the protein a-gold complex. The gold particle is found in layers lamina rara interna of glomerular basement membrane. (arrow) (x17,000)





**Figure 9** (From case No.PA10) Lupus nephritis class V, treated. A: Glomerular structure staining with H&E, LN class V, treated. (x40) Glomeruli are shows scleriosis. Most of them show moderate enlargement. Capillary wall thickness is slightly increased. Obliteration of the capillary lumens is not present. Endothelial cell and mesangial cell are unremarkable. Slightly segmental increase in mesangial cell and matrix are noted. No crescentic formation is seen. B: Immunofluorescence finding. (x40) Finely granular and discrete capillary wall deposits for IgG (3+), IgM(trace), IgA (negative)C3(trace), C4(negative), C1q(trace), kappa(2+), lambda(2+) and fibrin (trace) are noted. C: Glomerulus stain with laminin beta-1 antibody by immunohistochemistry (IHC) technique. (x40) GBM have positive staining, which is dyed with laminin beta-1. D: Electron microscopic finding. Using laminin beta-1 antibody and the protein a-gold complex. The gold particle is found in layers lamina densa of glomerular basement membrane. (arrow) (x17,000)



**Figure 10** (From case No.PA11) Lupus nephritis class V. A: Glomerular structure staining with H&E, LN class V. (x40) All of the glomeruli reveal mild enlargement with moderate capillary wall thickening. spikeformation can be identified by silver staining. Subendothelial deposits (wire-loop lesion) cannot be identified. Endothelial cell is not. B: Immunofluorescence finding. (x40) Gomeruli show diffuse uniform capillary wall deposits for IgG (3+), IgM (2+), IgA (2+), C3 (2+), C4 (1+), C1q (3+), kappa (2+), lambda(3+) and fibrin (negative) are noted. C: Glomerulus stain with laminin beta-1 antibody by immunohistochemistry (IHC) technique. (x40) GBM have positive staining, which is dyed with laminin beta-1. D: Electron microscopic finding. Using laminin beta-1 antibody and the protein a-gold complex. The gold particle is found in layers lamina rara interna of glomerular basement membrane. (arrow) (x17,000)

**Table 1** Show various class of Lupus nephritis and result EM localization

Case	Age	Sex	Various class of Lupus nephritis	EM localize
PA02	41	Female	Lupus nephritis class III (A/C) combined with Class V	GBM, Mesangial Matrix
PA03	39	Female	Lupus nephritis class III+V	GBM, Mesangial Matrix little
PA04	17	Female	Lupus nephritis class IV-G (A) with regression to class II	GBM, Mesangial Matrix
PA05	18	Female		
PA06	16	Female	Lupus nephritis class IV-G (A)	GBM, Mesangial Matrix
PA07	18	Female	Lupus nephritis class IV-G (A/C)	GBM, Mesangial Matrix
PA08	52	Female		
PA09	14	Female	Lupus nephritis class IV-G (A) plus class V	GBM, Mesangial Matrix
PA10	15	Female	Lupus nephritis class V, treated	GBM, Mesangial Matrix
PA11	19	Female	Lupus nephritis class V	GBM, Mesangial Matrix

## Discussion

The exact pathogenic processes accounting for development of glomerulonephritis in SLE have remained elusive. The extent to which immune complex deposits are derived from the circulation or are formed locally by the cross-reaction between anti-dsDNA and actin in or other glomerular constituents has been examined. Nucleosomes have been suggested as a major immunogen responsible for the induction of potentially pathogenic antinuclear antibodies and they also seem to serve as a target antigen complex for autoantibody-mediated tissue lesions in the kidneys of patients with lupus nephritis. The possibility that nucleosomes are trapped in the glomeruli by glomerular constituents such as type IV collagen, heparin sulfate, or other negatively charged residues has been suggested previously. Impaired clearance of apoptotic cells has been proposed as a defect that may explain a systemic and intra glomerular release of nucleosomes as a significant source of autoantigens (Anders I. Olin et al., 2014).

It has been shown that nucleosomes have the capacity to associate with the GBM or the mesangial matrix in murine and human variants of lupus nephritis. In glomerular membranes, they are consistently observed as small and larger EDDs, which are found both sub-endothelial and sub-epithelial. Olin show in the present study that at least one reason for the trapping could be that they have intrinsic affinity for specific GBM structures, as previously reported by Mjelle et al. Who demonstrate binding to a mixture of laminins and to isolated type IV collagen. The laminins form cruciform or T-like structures de-pending on their chain content and are held together with the type IV collagen meshwork by nitrogen and heparin sulfate proteoglycans. Laminins self-assemble, and they fully traverse the GBM. All of these characteristics, together with the known up-regulation of laminin during infection and inflammation, make them ideal ligands for the attachment of nucleosomes, which is relevant to the development of nephritis in SLE patients.

However, the normal adult laminin of the GBM, laminin111, does not bind nucleosomes. Olin et al were able to visualize the interactions between nucleosomes and laminin beta1-containing laminins by electron microscopy. In addition, the binding strength of these interactions was found to be substantial. which is consistent with study of Mjelle

et al. Individual chains did not bind to nucleosomes nor did the alpha-2 chain of laminin 2 bind to nucleosomes, whereas full-length laminin 2 did bind to nucleosomes. Since normal adult laminin 11 did not bind nucleosomes, Olin et al conclude that like the beta-2 chain, the gamma-1 chain could not be a determinant for the nucleosome binding.

Olin et al found that that laminin beta-1 chain with affinity for nucleosomes was found to be aberrantly expressed in the kidneys of patients with lupus nephritis, which is a novel finding in this disease, since laminin beta-1 is not normally expressed in adult and mature GBM. Nucleosomes and laminin beta-1-containing laminin colocalize in large and smaller electron dense deposits (EDDs) distributed along the membranes of the nephritic glomeruli in SLE patients. Sixt et al demonstrated that normally expressed laminin beta-2 did not colocalize with nucleosomes. Olin et al also confirmed this finding. In the same biopsy samples, Olin et al detected specific staining for TGF beta-1, and suggested that local production of TGF beta-1 by podocytes and mesangial cells drives the production of immature laminin chains. This feature has also been demonstrated experimentally using TGF beta-1 transgenic animals. TGF beta-1 induces the expression, and thus the deposition, of type IV collagen as well as aberrant fetal laminin alpha-1, alpha-2, and beta-1 chains into the GBM.

## Conclusion

Results from nine case, The sample study are age 14-52 years these all women of lupus nephritis, PA02, PA03, PA04, PA05, PA06, PA07, PA08, PA09, PA10, PA11 and one case control (PA01) in Srinagarind hospital. Khon Kaen, Thailand consists of Class III, IV and V by the Immuno-gold electron microscopic technique.

Laminin beta-1 was found in all classes but the difference was found. The most common class was Class IV, the lowest class III combined with Class V in position Glomerular basement membrane (GBM) and Mesangial matrix.

In conclusion, our findings are compatible with previous studies that nucleosomes derived from apoptotic cells are trapped within glomerular basement membranes during the development of lupus nephritis. Here, circulating anti-dsDNA antibodies may cause nephritis through binding the planted antigens in situ, although the possibility that the nucleosomes also could bind GBMs as part of preformed nephritogenic immune-complexes cannot be excluded and conclude that aberrant laminin beta-1 is an intrinsic ligand of the GBM, in feature could be of a significant pathobiologic interest during the development of lupus nephritis.

The results indicate that in Thai people, there is a mechanism similar to that in the previous reports. In addition, we also find that there is also aberrant expression of laminin beta-1 in Class III and Class V, and give us a further understanding that the mechanism of LN can be planted antigen immune complex formation, in addition to circulating immune complex formation.

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