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# “Evaluación de la Capacidad que tienen los Niveles de Acido Desoxirribonucleico Libre Circulante Mitocondrial y Nuclear Urinario para Discriminar Muestras de Sujetos con Nefritis Lúpica”.

**“Assessment of the Ability of Mitochondrial and Nuclear Urinary Cell Free Circulating Deoxyribonucleic Acid Levels to Discriminate Samples from Subjects with Lupus Nephritis”.**

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**RESUMEN**

**Antecedentes:** El lupus eritematoso sistémico (LES), es una enfermedad autoinmune, proclive a una presentación clínica heterogénea que debuta de manera silente en casos de nefritis lúpica activa (NLA), lo cual es mandatorio de una biopsia renal percutánea con consecuentes eventos adversos.

**Objetivo:** Evaluar la capacidad que tienen los niveles de ADN libre circulante mitocondrial y nuclear urinario (uADN-lc) para discriminar muestras de sujetos con nefritis lúpica activa (NLA).

**Sujetos y Métodos:** Se tomaron muestras de orina de (74) sujetos, (25) con LES sin compromiso renal, (12) con NLA (12) con NLR y (25) Controles sanos. Se realizó una reacción en cadena de la polimerasa en tiempo real (qPCR) para la cuantificación de las moléculas de ácido desoxirribonucleico libre circulante (ADN-lc), mediante el empleo de genes constitutivos ND1 (mitocondrial) y GAPDH (nuclear), a posteriori de su extracción urinaria (uADN-lc), mediante el Kit Comercial de Aislamiento con Perlas Magnéticas (*Applied Biosystems*), y la determinación de su concentración con espectrofotómetro *(Nanodrop One 2000 de Thermo Scientific ®)* y analizador de imágenes.

**Resultados:** La correlación entre grupos demostró que el grupo de LES presentó asociación con el parámetro de actividad (SLEDAI) y el grupo de NLA con la cronicidad (SLICC), cuyo ($p<0,05$) y un intervalo de confianza del 95%. En cuanto a la comparación de los niveles de (ADN-lc) entre los grupos se evidenció que el

grupo de LES presentó la mediana de mayor rescate con (10,10 ng/µl), y el grupo de Control registró una menor con 6,9 ng/µl, mientras que los grupos NLA, 8,05 ng/µl y el grupo NLR con 4,5250 ng/µl; respectivamente. La tendencia de las diferencias estadísticamente significativas ($p<0,05$) en los niveles de las concentraciones de (ADN-lc) se determinó entre los grupos LES y NLR con un ($p<0,037$). Entre tanto, el grupo NLA registró la mayor cuantificación del número de copias de (ADN-lc) para el gen ND1 con una mediana de 10144,82 copias/µl y la menor para el gen GAPDH en el grupo NLR con 27,32 copias/µl. La sensibilidad predictiva de nefritis lúpica fue del 68% para el gen GAPDH y del 58% para el gen ND1.

**Conclusión:** Existe tendencia estadísticamente significativa en los niveles de las concentraciones de (ADN-lc) mitocondrial y nuclear para discriminar muestras de orina de sujetos con nefritis lúpica activa (NLA). La cuantificación del número de copias/µl de (ADN-lc) fue mayor en el grupo de NLA, en el cual, el gen ND1 reportó una amplificación superior, cuya expresión representó a los anticuerpos Anti-ADN en respuesta al antígeno (ADN-lc) producto del origen apoptótico del tejido renal del cual provino la fuente mitocondrial a expensas de células mesangiales, previo al hallazgo histológico y a una replicación inferior que demostró el gen constitutivo GAPDH en el grupo de NLR, pero con una mayor sensibilidad para predecir NL, en cuyo caso, la reacción de anticuerpos anti nucleares (ANAs) se presentó frente al (ADN-lc) de procedencia nuclear oriundo de células podocíticas; constituyendo los complejos inmunes propios del LES.

**Palabras clave: Complejos inmunes, concentración de ácido desoxirribonucleico libre circulante urinario (uADN-lc), GAPDH, SLEDAI, SLICC, ND1, Nefritis lúpica activa (NLA).**

## ABSTRACT

**Background:** Systemic lupus erythematous (SLE) is an autoimmune disease, prone to a heterogeneous clinical presentation that debuts silently in cases of active lupus nephritis (ALN), which is mandatory for a percutaneous renal biopsy with consequent adverse events.

**Objective:** To evaluate the ability of mitochondrial and nuclear urinary cell-free DNA (cf-uDNA) levels to discriminate samples from subjects with active lupus nephritis (ALN).

**Subjects and Methods:** Urine samples were taken from (74) subjects, (25) with SLE without kidney failure, (12) with (ALN), (12) with (RLN) and (25) Healthy Controls. A quantitative real-time polymerase chain reaction (Q-PCR) was performed for cell-free deoxyribonucleic acid molecule quantification (cf-DNA), using constitutive genes ND1 (mitochondrial) and GAPDH (nuclear), after their urinary extraction (cf-uDNA), by means of the Commercial Isolation Kit with Magnetic Pearls (*Applied Biosystems*), and its concentration measurement with spectrophotometer (*Nanodrop One 2000 de Thermo Scientific* ®) and image analyzer.

**Results:** The correlation between groups showed that SLE group presented association with the activity parameter (SLEDAI), and the (ALN) group, with the chronicity (SLICC), whose ($p<0.05$) with a confidence interval of 95%. The comparison of (cf-DNA) levels between the groups revealed that the SLE group had the highest median rescue with (10.10 ng/µl), and the Control group had a lower one with (6.9 ng/µl), while the NLA groups, (8.05 ng/µl) and the NLR group (4.5250 ng/µl); respectively. The trend in statistically significant differences ($p<0.05$) in the levels of (cf-DNA) concentrations was established between the SLE and RLN groups with ($p<0.037$). Meanwhile, the NLA group recorded the highest quantified number of copies for (cf-DNA) whose median for the ND1 gene was 10144.82 no. of copies/µl and the lowest no. copies/µl for the GAPDH gene in the NLR group with 27.32. The predictive sensitivity for lupus nephritis was 68% for the GAPDH gene and 58% for the ND1 gene.

**Conclusion:** There is statistically significant trend in the levels of mitochondrial and nuclear (cf-DNA) concentrations to discriminate urine samples from subjects with active lupus nephritis (ALN). The number of copies/µl quantified for (cf-DNA) was higher in the (ALN) group, in which the ND1 gene reported a superior amplification, whose expression represented Anti-DNA antibodies in response to the antigen (cf-DNA), product that arose from kidney apoptotic origin from which the mitochondrial source came from, in expense from mesangial cells, prior to the histological onset and a lower replication seen with the constitutive gene GAPDH in the RLN group, in which case, the anti-nuclear antibody (ANAs) reaction was

presented upon (cf-DNA) from nuclear provenance from native podocyte cells; constituting the immune complexes typical of SLE.

**Keywords: SLEDAI, SLICC, concentration of urinary circulating free deoxyribonucleic acid (uDNA-lc), GAPDH, ND1, Active lupus nephritis (ANL).**

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