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Circulating Microrna (148b And 150) as Potential Biomarker in Iga Nephropathy and Lupus Nephritis

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Abstract:

Background: IgA nephropathy (Berger's disease or mesangial IgA deposition) is characterized by deposition of the IgAauto-antibody in the glomerulus causes a variety of lesions and inflammation in renal tissue. The clinical course of IgA nephropathy is usually mild, although in 40% of the cases it may lead to end-stage renal disease and it is considered is the most common type of primary GN in the world.

One of the mostfrequent organ manifestations of SLE is LN, The disease most affected patients are women of childbearing age, and it is characterized by overproduction of antibodies to self-antigens, which are mostly extracted from cell components including the nucleus, cell membranes, cytoplasm, and ribosomes.which in many cases leads to end-stage renal disease.

Objectives: biopsy is the best way to diagnose patients with IgA nephropathy and Lupus nephritis But it is considered an invasive method for diagnosis, therefore the current study recommends that miRNA-148b and miRNA-150 be evaluated as biomarkers for IgA nephropathy and Lupus nephritis diagnosis.

Material and Methods

study the control cases in three groups, first group consist from 30 patients previously diagnosed as IgA Nephropathy(IgAN) which include (19 male and 11 female), also Second group was 30 patients who have Lupus Nephritis(LN) which include (10 male and 20 female) who were under the supervision of nephrology specialists at Al Hussein Teaching Hospital in Holy Karbala province from December 2019 to December 2020. Thirty healthy volunteers (non-IgA nephropathy and non-Lupus nephropathy) were included in the third group.

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The blood sample were collected by venipuncture from three groups were drawing approximately five millimeter of venous blood by disposable syringe under aseptic technique ,Each blood sample of these groups were collected in plane tube then serum was separated by centrifugation 13000 for 5 minute.Complete RNA was extracted from serum samples using the (TRIzol® reagent kit) after the serum was collected in tubes and stored at -20°C. Bioneer. Korea) and completed in accordance with company guidelines.

Result: Comparison of miR-148b was highly significant difference in gene expression among study groups ($p < 0.001$); the level being highest in lupus nephritis followed by IgA nephropathy and then by control group.Comparison of miR-150was highly significant difference in gene expression among study groups ($p < 0.001$); the level being highest in IgA nephropathy followed by lupus nephritis and then by control group.A receiver operator characteristic (ROC) analysis; Regarding IgA nephropathy , the results showed that the cutoff values for miR-148b, miR-150 were >1.69, >5.53 respectively;The most accurate one was miR-150 followed by miR-148b with accuracy levels of 100.0 %and 75.1%, respectivelywithpoor sensitivity and specifity for miR-148b were 73.3% and 70.0% respectively,while excellent miR-150 the sensitivity and specifity were 100.0% and 100.0% respectively.

.whileRegarding lupus nephritis , the results showed that the cutoff values for miR-149b, miR-150 were >1.69, >3.55 respectively; the most accurate one was miR-148b followed by miR-150 with accuracy levels of 91.7 % and 89.4 % respectively,with sensitivity and specifity for miR-148b were 100.0 % and 70.0% respectively,while excellent miR-150 the sensitivity and specifity were 80.0% and 90.0% respectively.

Conclusion: Present study concluded that, moRNA-150 consider a good biomarker with high sensitivity and specificity for IgA nephropathy, while miRNA-148b could serve a diagnostic biomarker with high sensitivity and specificity for Lupus nephritis.

Key words: miRNA-148b,miRNA-150,glomerulonephritis,IgAN, LN. Biomarker

Introduction

IgA nephropathy (Berger's disease or mesangial IgA deposition) is characterized by deposition of the IgA auto-antibody in the glomerulus causes a variety of lesions and inflammation in renal tissue(1). The clinical course of IgA nephropathy is usually mild, although in 40% of the cases it may lead to end-stage renal disease and it is considered is the most common type of primary GN

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in the world (2). Genome-wide association studies (GWAS) have domenstrated a number of multiple genomic polymorphisms which related to susceptibility such as those involved in the response to pathogens and associations of IgAN with several single-nucleotide polymorphisms within or near immune-related genes such as major histocompatibility (MHC)loci (3),(4).

One of the mostfrequent organ manifestations of SLE is LN, The disease most affected patients are women of childbearing age(5), and it is characterized by overproduction of antibodies to self-antigens, which are mostly extracted from cell components including the nucleus, cell membranes, cytoplasm, and ribosomes.which in many cases leads to end-stage renal disease(6).

MicroRNAs are a broad family of RNAs that are 21–22 nucleotide long and have emerged as important post-transcriptional regulators of gene expression in plants and animals.MicroRNAs are thought to regulate the function of ~50% of all protein-coding genes in animals (7).

miRNAs are also play an essential role in the pathogenesis of GN includingIgANand LN by Immune complexes are deposited or produced in situ in the kidney, triggering cytokine and chemokine responses and causing damage to the endothelium of the glomerular capillary loops, podocytes, small renal vessels, tubules, and renal interstitium(8).IgA Nephropathy and Lupus Nephritis patients have kidney lesions in approximately 50% of patients. Not only were variations in abundance of serum circulating microRNAs in LN measured in a study by Navarro-Quiroz et al., but specific microRNAs were also evaluated as possible contributors to LN pathogenesis(9).Perhaps in the future, MiRNAs can become therapeutic targets for IgANand LN pateints, in addition to their function in the diagnosis of renal diseases (10).

Thus, according to such contraversary the aims of present study are :To find out the clinical importance of miRNA-148b ,miRNA-150 and miRNA-155 as diagnostic biomarker for IgA Nephropathy and Lupus Nephritis .

Materials and Methods

study the control cases in three groups, first group consist from 30 patients previously diagnosed as IgA Nephropathy(IgAN) which include (19 male and 11 female), also Second group was 30 patients who have Lupus Nephritis(LN) which include (10 male and 20 female) who were

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Total RNA Extraction

Total RNA was extracted according to the manufacturer's instructions using the TRIzol® reagent kit (Bioneer, Korea). The check extracted genomic DNA was using a nanodrop spectrophotometer (THERMO, USA). Reading the absorbance at 260/280 nm was used to measure DNA purity and concentration.

Stem loop Real time-PCR

The stem loop Real time-PCR was used in quantification of miRNA-148, miRNA-150 in expression analysis that normalized by housekeeping gene (GAPDH) in normal samples and serum patients by using Real-Time PCR technique and this procedure was carried out according to the stated method(11).

Probes and Primers

Probes and Primers for the GAPDH gene

The primer three designs are available online, as well as NCBI-GenBank.The GAPDH gene probes and primers were designed using the database. All of these primers were provided by the Macrogen Company in Korea, as shown in the table below:

Gene	**Sequence**
GAPDH-probe	FAM-CCAGCCGAGCCACATCGCTC-TAMRA
GAPDH –primer	F : TCAGCCGCATCTTCTTTTGC R : TTAAAAGCAGCCCTGGTGAC

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miRNA probes and primers

The design of probes and primers for miRNA-148, miRNA-150 was done in recent research to determine miRNA sequence while using miRNA primer design tool by the sanger center miRNA database registry. Macrogen Company, Korea delivered these probes and primers as given in the table below.

PRIMER	SEQUENCE	AMPLICON
RT primer (specific) hsa-miR-148b	F:GTCGTATCCAGTGCAGGGTCCGAGG R:TATTCGCACTGGATACGACGCCTGA	
hsa-miR-148b qPCR primer	F:AGCCAGCGAAGTTCTGTTATAC R: GTCGTATCCAGTGCAGGGT	72bp
RT primer (specific) hsa-miR-150	F:GTCGTATCCAGTGCAGGGTCCGAGG R:TATTCGCACTGGATACGACCTGTCC	
miR-150qPCR primer	F:AACAAGCTGGTACAGGCCT R:GTCGTATCCAGTGCAGGGT	72bp

qRT-PCR data analysis

results of qRT-PCR data for thehousekeeping gene and miRNAwere analyses with help of relative quantitative levels of fold change (gene expression). therefore , the ΔCT method was chosen while using the reference called by Scmittgenand Livak(12)as following equations:

Ratio (reference/target) = 2CT(reference)–CT(target)

Result

miR-148, miR-150 and miR-155 gene expression in patients and control subjects

Comparison of miR-148b is shown in table 1 and figure 1. There was highly significant difference in gene expression among study groups ($p < 0.001$); the level being highest in lupus nephritis followed by IgA nephropathy and then by control group.

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Comparison of miR-150 is shown in table 1 and figure 2. There was highly significant difference in gene expression among study groups ($p < 0.001$); the level being highest in IgA nephropathy followed by lupus nephritis and then by control group.

Table 1: miR-148, miR-150 gene expression in patients and control subjects

Characteristic	**Control** ***n*** **= 30**	**IgA nephropathy** ***n*** **= 30**	**Lupus nephritis** ***n*** **= 30**	***P***
miR-148b				
Median (IQR)	0.98 (3.07) C	3.92 (11.07) B	9.29 (17.17) A	<0.001 K HS
Range	7.83 -0.10	248.79 -0.41	281.85 -1.81	
miR-150				
Median (IQR)	1.05 (2.15) C	30.57 (17.61) A	8.08 (9.79) B	<0.001 K HS
Range	5.53 -0.10	67.35 -11.82	35.59 -0.58	

n: number of cases; **IQR**: Inter-quartile range; **K**: Kruskal Wallis test; Capital letters (A, B and C) were used to indicate the level of significance following **Mann Whitney U** test so that A indicate the highest value followed by B and then C and that different letters indicate significant difference at $p \leq 0.05$; whereas, same letters indicate no significant difference at $p > 0.05$; **HS**: highly significant at $p < 0.001$

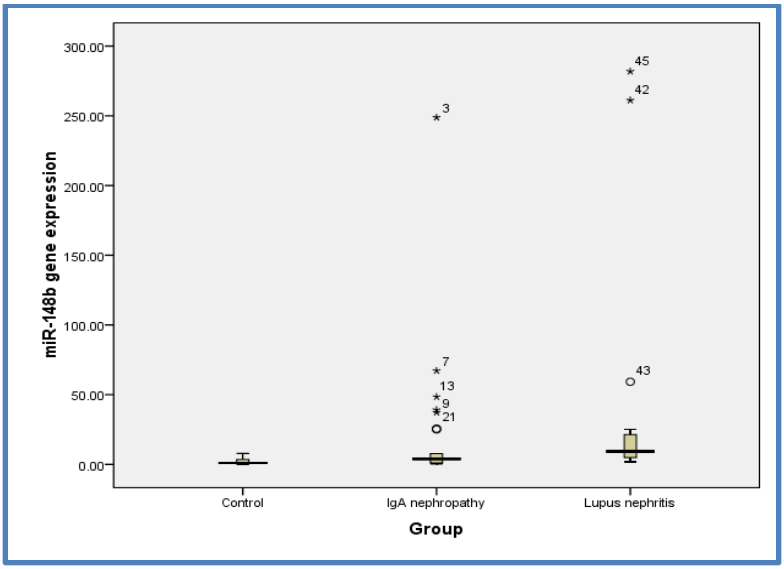

Figure 1: Box plot showing comparison of miR-148 gene expression in patients and control subjects

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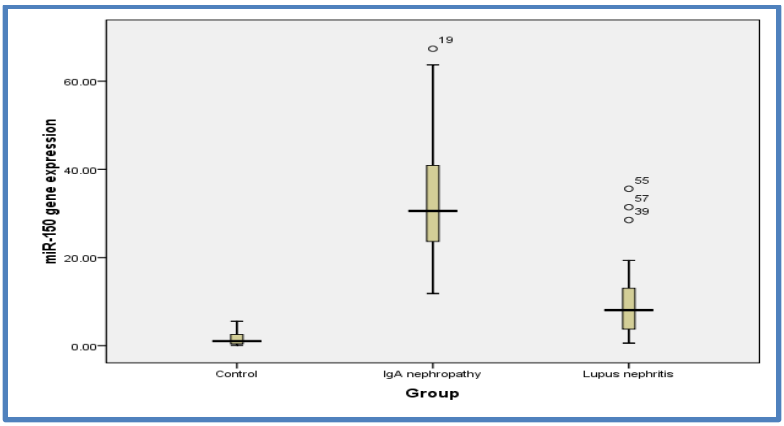

Figure 2: Box plot showing comparison of miR-150 gene expression in patients and control subjects

In order to figure out the diagnostic role of miR-gene expression in IgA nephropathy and lupus nephritis receiver operator characteristic curve (ROC) analysis was carried out and the results are shown in tables 2 through figures 3and figures4.

Regarding IgA nephropathy (taking IgA nephropathy as single group and making a contrast with control group) the results showed that the cutoff values for miR-149b, miR-150 were >1.69, >5.53 respectively; the most accurate one was miR-150 followed by miR-148b with accuracy levels of 100.0 %and 75.1% respectivelywith sensitivity and specifity for miR-148b were 73.3% and 70.0% respectively,while excellent miR-150 the sensitivity and specifity were 100.0% and 100.0% respectively., table 2 and figures 3and 4

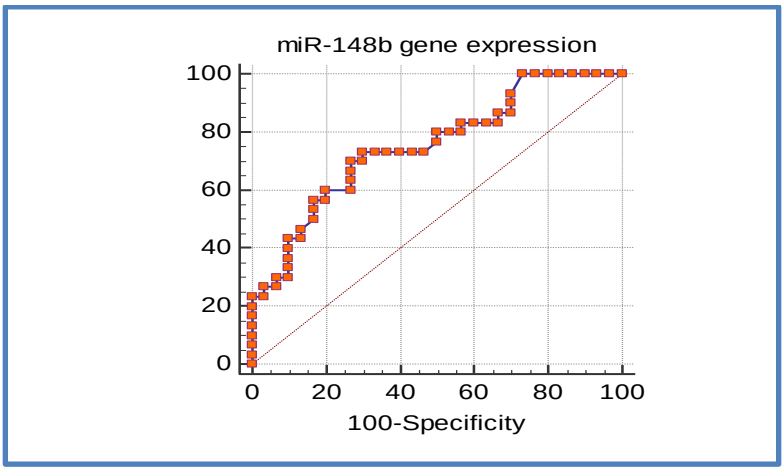

Figure 3: Receiver operator characteristic (ROC) curve analysis to find the cutoff value of miR-148b that predict a diagnosis of IgA nephropathy with best accuracy

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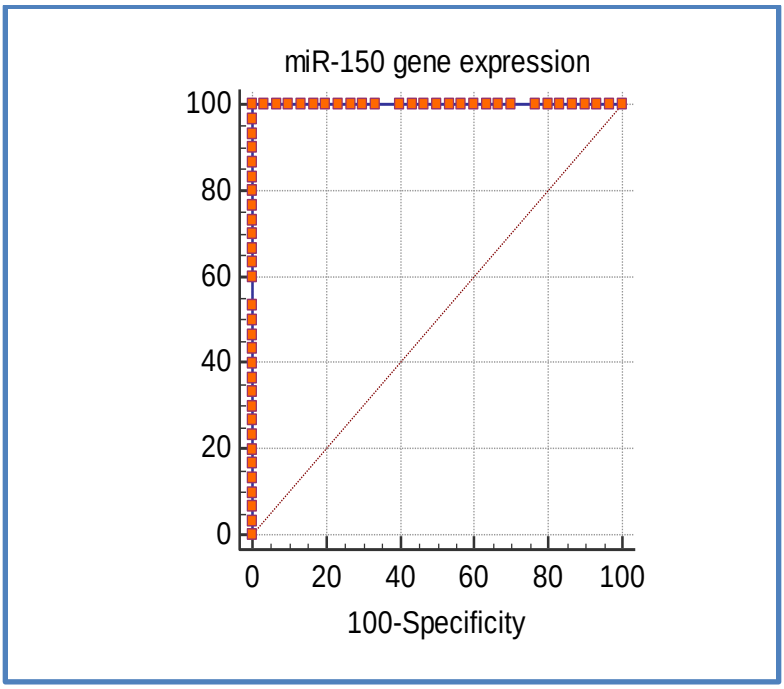

Figure 4: Receiver operator characteristic (ROC) curve analysis to find the cutoff value of miR-150 that predict a diagnosis of IgA nephropathy with best accuracy

Table 2: The characteristics of ROC curve when IgA nephropathy group where compared to control group

Characteristic	**miR-148b**	**miR-150**
Cutoff	>1.69	>5.53
AUC	0.751	1
95 % CI	0.623 to 0.854	0.940 to 1.000
Accuracy %	75.1	100
p-value	< 0.001	< 0.001
Sensitivity %	73.3	100.0
Specificity %	70.0	100.0

AUC: area under curve; **CI**: confidence interval; **HS**: highly significant

while Regarding lupus nephritis , the results showed that the cutoff values for miR-148b, miR-150 were >1.69, >3.55 respectively; the most accurate one was miR-148b followed by miR-150 with accuracy levels of 91.7 % and 89.4 % respectively.with sensitivity and specifity for miR-

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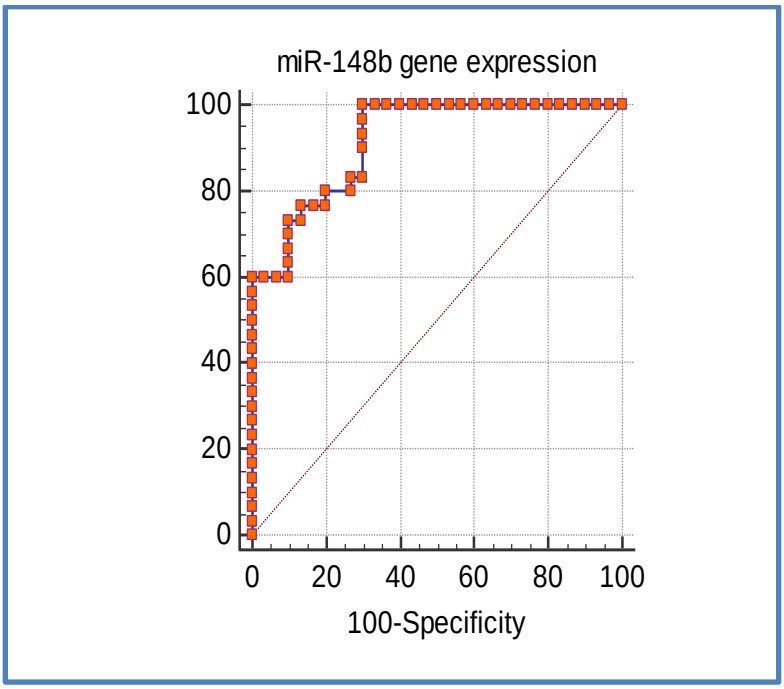

Figure 5: Receiver operator characteristic (ROC) curve analysis to find the cutoff value of miR-148b that predict a diagnosis of lupus nephritis with best accuracy

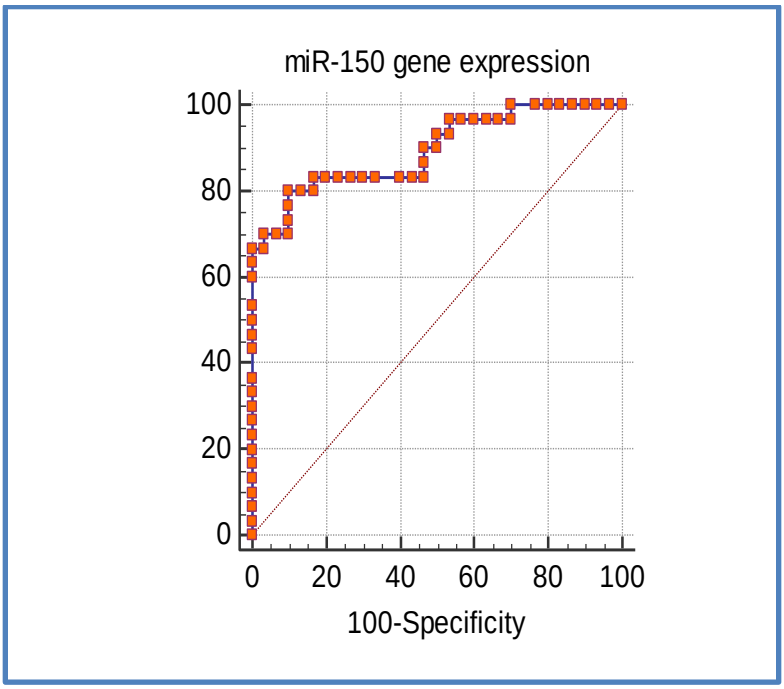

Figure 6: Receiver operator characteristic (ROC) curve analysis to find the cutoff value of miR-150 that predict a diagnosis of lupus nephritis with best accuracy

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Table 3: The characteristics of ROC curve when lupus nephritis group where compared to control group

Characteristic	miR-148b	miR-150
Cutoff	>1.69	>3.55
AUC	0.917	0.894
95 % CI	0.816 to 0.972	0.788 to 0.959
Accuracy %	91.7	89.4
p-value	<0.001	<0.001
Sensitivity %	100.0	80.0
Specificity %	70.0	90.0

AUC: area under curve; **CI**: confidence interval; **HS**: highly significant

DISCUSSION:

A study that has been done by Chandrasekaran et. al found that miRNAplays an increasingly important regulatory function in the growth, physiology, and maintenance of adult-kidney microstructure in a study. Though the expression profiles of miRNAs in various renal diseases have been studied, further research is required to fully comprehend the functions of miRNA in renal pathophysiology(13).

MiRNA-148b also play roles in a mesangial and endothelial cell which are two glomerular cell types that interact with Podocytes then leading to defect in the work of kidney and development the pathogenesis of LN in patients (14). Through previous studies have shown that the miRNA affect the physiology of disease for a range of diseases, the most important kidney disease through its impact on Nephron and then can be indicative of its presence in the diagnosis of diseases GN(15),(16). MiRNA-148b also involved in the physiology of the pathogenesis of many types of cells, especially the podocyte Through Dicer targeted deletion. The Dicer is the important enzyme in the synthesis or biogenesis of miRNA Thus resulting in it appear the protein in the urine (proteinuria), podocyte injury, glomerulosclerosis and finally leading to end stage of the renal disorder (ESRD) (17).

Many studies have shown a critical role for miR-148b in developing kidney with its physiologyand pathophysiology, The miR-148b also play roles in a mesangial and endothelial

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cell which are two glomerular cell types that interact with Podocytes then leading to defect in the work of kidney and development the pathogenesis of LN in patients through miR-148b stimulated to renal fibrosis by downregulatingSuppressor of cytokine signaling 1(SOCS1) through in vitro study (18). Several studies have shown that a miR-148bare specific to certain kidney tissues or stages of development, showed that it is up-regulated in both mouse and human mesengialcellsand also increased mainly in renal tubular cells and podocytes in the repeated renal biopsies of flared American LN patients (19). Following our findings, other researchers discovered that depleting miR-148b reduces renal tubulointerstitial fibrosis in mice 8 weeks after I/R by growing collagen form I and connective tissue growth factor (20).Besides kidney diseases, miR-148b level increased in glomerulonephritis patientsespeciallyLN compared to those withSjogren's syndrome, celiac disease ,cardiovascular diseases, diabetes mellitus, hypertension,breast cancer, pancreatic Cancer(21) in another study showed that urinary miR-148b level alone has the best diagnostic accuracy,it is considered that miR-148b might be playing a role in the pathogenesis of LN and have the potential to be used as a diagnostic and prognostic marker for the prediction of LN. In this study, we have tried to find the diagnostic and prognostic importance of miR-148b for LN(22).

a study done by (Bezman et al.) they observed that glomerular up regulation of miR-150 has been detected in IgAN, The C1GALT1 gene has been shown to be inhibited by overexpression of miR-150 in peripheral blood mononuclear cells. Patients with IgA nephropathy had lower C1GALT1 expression, which was negatively associated with miR-150 expression. MiR-150 binds to C1B3GALT mRNA's 3' untranslated region and breaks it down. IgAN patients have substantially higher levels of miRNA-150 expression. The Th2 cytokine, IL-4, may downregulate C1-3GALT, and the Th2 cytokine, miR-150, may be induced by the Th2 cytokine (23).

Overexpression of miR-150 can cause massive proteinuria, in which significant quantities of protein are removed from the bloodstream, by disrupting the filtration slits or destroying the Podocytes. Proteinuria is a symptom of this condition, which leads to end-stage renal failure. also any defect in filtration will leading to problem in urea metabolism because the urea is freely filtered by the glomerulus and then passively reabsorbed in both the proximal and distal nephrons (24).

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Another study recently found that miR-150 expression may be a useful target for preventing or slowing the progression of IgAN through various pathways.The remarkable stability of circulating miR-150 has made them valuable for use as novel biomarkers in in IgAN. Chandrasekaran et al. discovered a variety of unusual miR-150 signatures associated with human kidney diseases, including IgAN,in various kidney diseases, miR-150 is expressed endogenously in the kidney, and some have been found to be upregulated in renal tissue and considered tissue specific in various kidney diseases (25).while found downregulated in other disease such as atherosclerosis, hypertension, diabetes mellitus, neuronal cell death, Prostate Cancer, Acute Myeloid Leukemia, Moreover, the authors suggested that MiR-150 has the ability to be used as a diagnostic marker for IgAN (26).

One research published in 2020 found that four miRs (135a-5p, 146b-5p, 150-5p, and 155-5p) are potential mediators of progression in IgAN and could be used as additional biomarkers for risk prediction in the future. In the ROC study, miR-150-5p was also found to be strongly expressed in kidney biopsies of IgAN patients who had advanced, and it showed strong discrimination between IgANp and IgANnp.expected , miR-150-5p may be a potential functional mediator of kidney fibrosis (27).

The present study also came in agreement with previous study conducted by (Kuwagata et al.) as it showed that there was high specifity and sensitivity for miR-148b in SLE and Several researches gives an evidence suggested that miR-148b play crucial role in SLE and participate in the development of LN and its complication (28). Another study done by Shumnalieva et al,. (2018) demonstrated there was low specifity and sensitivity for miR-155 .

The expression levels of serum miR-148b could differentiate SLE patients from healthy controls with area under curve(AUC)=0.711 (95% CI: 0.585÷0.837,p=0.002) according to the ROC curve study. MiR-155 had a lower diagnostic precision, with an AUC of 0.691 (95% CI: 0.566÷0.817, p=0.005) and.

miR-148b levels are significantly different between SLE and healthy controls; serum miR-148b level alone has the best diagnostic accuracy(29).

miR-148b higher sensitivity and specificity may be explained by a previous study that found that miR-148b is significantly up-regulated in podocytes in lupus nephritis, where it specifically targets the expression of WT1, a crucial transcription factor for podocyte differentiation and

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health.Surprisingly, extracellular miRNAs are abundant in blood and other biological fluids(30) .Globally, the increased prevalence of glomerulonephritis, especially IgAN and LN, has resulted in higher morbidity and mortality rates.Early detection of IgA nephropathy and lupus nephritis patients is critical in order to implement timely treatments, prevent kidney failure, and improve prognosis (25). Identifying true, non-invasive, responsive, and precise biomarkers that correlate well with kidney pathology and disease progression is thus critical. However, current kidney function tests such as blood urea nitrogen (BUN), serum creatinine, and proteinuria seem to be insufficient (31).

Conclusion: Present study concluded that, moRNA-150 consider a good biomarker with high sensitivity and specificity for IgA nephropathy, while miRNA-148b could serve a diagnostic biomarker with high sensitivity and specificity for Lupus nephritis.

Refereneces

1. Manno C, Bonifati C, Torres D, Campobasso N, Schena F (2011). Desmopressin acetate in percutaneous ultrasound-guided kidney biopsy: a randomized controlled trial. *American Journal of Kidney Diseases.* 57: 850–855 .

2. Gharavi A, Kiryluk K, Choi M, Li Y, Hou P, Xie J, Sanna-Cherchi S, Men C, Julian B, Wyatt R, Novak J, He J, Wang H, Lv J, Zhu L, Wang W, Wang Z, Yasuno K, Gunel M, Mane S, Umlauf S, Tikhonova I, Beerman I, Savoldi S, Magistroni R, Ghiggeri G, Bodria M, Lugani F, Ravani P, Ponticelli C, Allegri L, Boscutti G, Frasca G, Amore A, Peruzzi L, Coppo R, Izzi C, Viola B, Prati E, Salvadori M, Mignani R, Gesualdo L, Bertinetto F, Mesiano P, Amoroso A, Scolari F, Chen N, Zhang H, Lifton R (2011). Genome-wide association study identifies susceptibility loci for IgA nephropathy. *Nature Genetic.* 43(4): 321–327.

3. Kiryluk K, Li Y, Sanna-Cherchi S, Rohanizadegan M, Suzuki H, Eitner F, Snyder H, Choi M, Hou P, Scolari F, Izzi C, Gigante M, Gesualdo L, SavoldiS, Amoroso A, Cusi D, Zamboli P, Julian B, Novak J, Wyatt R, Mucha K, Perola M, Kristiansson K, Viktorin A, Magnusson P, Thorleifsson G, Thorsteinsdottir U, Stefansson K, Boland A, Metzger M, Thibaudin L, Wanner C, Jager K, Goto S, Maixnerova D, Karnib H, Nagy J, Panzer U, Xie J, Chen N, Tesar V, Narita I, Berthoux F, Floege J, Stengel B, Zhang H, Lifton R, Gharavi A(2012). Geographic differences in genetic susceptibility to IgA nephropathy: GWAS replication study and geospatial risk analysis. *PLoS Genetics.* 8(5): e1002765

4. Feehally J, Farrall M, Boland A, Gale D, Gut I, Heath S, Kumar A, Peden J, Maxwell P, Morris D, Padmanabhan S, Vyse T, Zawadzka A, Rees A, Lathrop M, Ratcliffe P (2010).

HLA has strongest association with IgA nephropathy in genome-wide analysis. *Journals of the American Society of Nephrology*. 21(10):1791–1797.

5. Yu X, Li M, Zhang H, Low H, Wei X, Wang J, Sun L, Sim K, Li Y, Foo J, Wang W, Li Z, Yin X, Tang X, Fan L, Chen J, Li R, Wan J, Liu Z, Lou T, Zhu L, Huang X, Zhang X, Liu Z, Liu J (2011). A genome-wide association study in Han Chinese identifies multiple susceptibility loci for IgA nephropathy. *Nature Genetics*. 44(2):178–182.

6. Tsai L, Hsiao S, Tsai L, Lin C, Tsai J, Liou D, Lan J (2011). The sodium-dependent glucose cotransporter SLC5A11 as an autoimmune modifier gene in SLE. Tissue Antigens 71: 114–126.

7. Rajewsky N. microRNA target predictions in animals. Nat Genet.2006;38 Suppl:S8-13.

8. Bentwich I, Avniel A, Karov Y, Aharonov R, Gilad S, Barad O, Barzilai A, Einat P, Einav U, Meiri E, Sharon E, Spector Y, Bentwich Z (2005). Identification of hundreds of conserved and nonconserved human microRNAs. *Nature Genetics.* 37(7): 766-770.

9. Navarro-Quiroz E, Pacheco-Lugo L, Navarro-Quiroz R, Lorenzi H, España-Puccini P, Díaz-Olmos Y, Almendrales L, Olave V, Gonzalez-Torres H, Diaz-Perez A, Dominguez A, Iglesias A, García R, Aroca-Martinez G (2017). Profiling analysis of circulating microRNA in peripheral blood of patients with class IV lupus nephritis. *PLoS One.* 12(11): e0187973.

10.Zhou H, Hasni S, Perez P, Tandon M, Jang S, Zheng C, Kopp J, Austin H, Balow J, Alevizos I and Illei G (2013). miR-150 Promotes Renal Fibrosis in Lupus Nephritis by Downregulating SOCS1.*American Society of Nephrology.* 24 (7) 1073-1087.

11. Varkonyi-Gasic E, Hellens RP. Quantitative stem-loop RT-PCR for detection of microRNAs. Methods MolBiol 2011;744:145-57.

12. Livak KJ, Scmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT Method. Method 2001;25:402-8.

13. Chandrasekaran K, Karolina D, Sepramaniam S, Armugam A, Wintour E, Bertram J. Jeyaseelan K (2012). Role of microRNAs in kidney homeostasis and disease. *Kidney International*. 81: 617–627.

14. Pollak, M. R. (2002). "Inherited podocytopathies: FSGS and nephrotic syndrome from a genetic viewpoint." Journal of the American Society of Nephrology **13**(12): 3016-3023.

15.Ventura, A., A. G. Young, M. M. Winslow, L. Lintault, A. Meissner, S. J. Erkeland, J. Newman, R. T. Bronson, D. Crowley, J. R. Stone, R. Jaenisch, P. A. Sharp and T. Jacks (2008). "Targeted deletion reveals essential and overlapping functions of the miR-17 through 92 family of miRNA clusters." Cell **132**(5): 875-886.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 6294 - 6309
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16. Wei C, Cheung W, Heng C, Arty N, Chong S, Lee B, Puah K, Yap H (2005) Interleukin-13 genetic polymorphismsin Singapore Chinese children correlate with long-termoutcome of minimal-change disease.*Nephrol Dial Trans-plant.* 20(4): 728–734.

17. Shi, S., L. Yu, C. Chiu, Y. Sun, J. Chen, G. Khitrov, M. Merkenschlager, L. B. Holzman, W. Zhang, P. Mundel and E. P. Bottinger (2008). "Podocyte-selective deletion of dicer induces proteinuria and glomerulosclerosis." J Am SocNephrol**19**(11): 2159-2169.

18. Pollak, M. R. (2002). "Inherited podocytopathies: FSGS and nephrotic syndrome from a genetic viewpoint." Journal of the American Society of Nephrology **13**(12): 3016-3023.

19. Nie F, Liu T, Zhong L, Yang X, Liu Y, Xia H, Liu X, Wang X, Liu Z, Zhou L, et al. MicroRNA-148b enhances proliferation and apoptosis in human renal cancer cells via directly targeting MAP3K9. Mol Med Rep. 2016; 13(1):83–90.

20. Serino G, Sallustio F, Cox S, Pesce F, Schena F (2012). Abnormal miR-148b expression promotes aberrant glycosylation of IgA1 in IgA nephropathy. *Journal of the American Society of Nephrology.* 23: 814–824.

21. 31. Szeto CC, Li PK. MicroRNAs in IgA nephropathy. Nat Rev Nephrol. 2014;10: 249–56.

22. Guan H, Peng R, Mao L, Fang F, Xu B, Chen M (2020). Injured tubular epithelial cells activate fibroblasts to promote kidney fibrosis through miR-150-containing exosomes. *Experimental Cell Research.* 392: 11-2007

23. Bezman N, Chakraborty T, Bender T, Lanier L (2011). miR-150 regulates the development of NK and iNKT cells. *Journal of Experimental Medicine*. 208(13):2717–31.

24. Löwik, M. M., P. J. Groenen, E. N. Levtchenko, L. A. Monnens and L. P. van den Heuvel (2009). "Molecular genetic analysis of podocyte genes infocal segmental glomerulosclerosis—a review." Eur J Pediatr**168**(11): 1291-1304.

25. Chandrasekaran K, Karolina D, Sepramaniam S, Armugam A, Wintour E, Bertram J. Jeyaseelan K (2012). Role of microRNAs in kidney homeostasis and disease. *Kidney International*. 81: 617–627.

26. Djudjaj, S., and Boor, P. (2019). Cellular and molecular mechanisms of kidney fibrosis. Mol. Aspects Med. 65, 16–36.

27. Pawluczyk ZA. Izabella, AthanasiosDidangelos, Sean J. Barbour, Lee Er, Jan U. Becker, Roberto Martin, Scott Taylor, Jasraj S. Bhachu, Edward G. Lyons, Robert Jenkins, Donald Fraser, Karen Molyneux, Javier Perales-Patón, Julio Saez- Rodriguez, Jonathan Barratt, Differential expression of microRNA miR-150-5p in IgA nephropathy as a potential mediator and marker of disease progression.2020 ,31553-2.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 6294 - 6309
Received 25 April 2021; Accepted 08 May 2021.

28. Kuwagata S, Kume S, Chin-Kanasaki M, Araki H, Araki S, Nakazawa J, Sugaya T, Koya D, Haneda M, Maegawa H, et al. MicroRNA148b-3p inhibits mTORC1-dependent apoptosis in diabetes by repressing TNFR2 in proximal tubular cells. Kidney Int. 2016;90(6):1211–25.

29. Shumnalieva R, Kachakova D, Shoumnalieva-Ivanova V, Miteva P, Kaneva R, Monov S (2018). Whole peripheral blood miR-146a and miR-155 expression levels in systemic lupus erythematosus patients. *ActaReumatológica Portuguesa*. 43(3):217-225.

30. Wasung M, Chawla L, Madero M (2015). Biomarkers of renal function, which and when? *ClinicaChimicaActa*. 438: 350–357.

31. Rysz J, Gluba-Brzozka A, Franczyk B, Jabłonowski Z, Ciałkowska-Rysz A (2017). Novel biomarkers in the diagnosis of chronic kidney disease and the prediction of its outcome. *International Journal of Molecular Sciences*. 18(8): 1702.