Nawal Khinteel Jabbar et al /J. Pharm. Sci. & Res. Vol. 10(11), 2018, 2960-2963

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

miRNA-146a expression as anti-inflammatory marker in Diabetic Nephropathy

Nawal Khinteel Jabbar[1], Anwar Jasib Almzaiel[2*], Ali Fawzi Abd Alsahib[3]

[1]*Department of Chemistry, College of science, AL-Qadisiyah University, Al-Dywaniyah, Iraq*
[2]*Department of Medical Chemistry, College of Medicine, AL-Qadisiyah University, Al-Dywaniyah, Iraq*
[3]*Al-Dywaniyah Education Hospital , Al-Dywaniyah, Iraq*

Abstract

Recent evidences have been demonstrated that micro RNA (miRNA) a small non coding RNA is involved in development of diabetic nephropathy (DN). Inflammation has been suggested to be a contributor. However, the role of miRNA in regulation of inflammatory response during DN still unclear, the study was aimed to characterize the expression of miRNA 146a in DN.

Total of30 patients with Typ2 DM, 30 patients with DN and 30 healthy controls were enrolled inthis study. HbA1C, serum creatinine, blood urea, serum albumin levels and superoxide dismutase (SOD) activity were determined by colorimetric method. TNF-α level was measured by ELISA and qPCR was employed to screen the expression of serum miRNA 146a.

The results showed a significant decrease in SOD activity ($P < 0.05$) in DN compared with DM and control groups. TNF-α levels were significantly increased compared to other groups ($P < 0.05$). The expression of miR-146a was significantly decreased in DN compared to DM and control groups ($P < 0.05$). The study concluded that decrease expression of anti-inflammatory miR-146a exerts anti-protective effect by upregulating target genes related to inflammation, and hyperglycemia induced oxidative stress taken together, the results identify the regulatory role of miRNA-146a in DN.

Keywords: Diabetic nephropathy, miRNA, oxidative stress, inflammation, hyperglycemia.

INTRODUCTION

Diabetic nephropathy (DN) is a main cause of end stage renal disease (ESRD) in developed world, and it is one of common chronic complications of DM. DN is described by elevated glomerular permeability to proteins and gradual kidney function decreased through "glomerular podocytes injury, glomerular basement membrane thickening, expansion of mesangial, endothelial dysfunction and fibrosis of tubule-interstitial" the main changes in DN [1], which clinically established as proteinuria and a steady deterioration in glomerular filtration rate [2].

Several factors may be involved in the pathogenesis of DN and its prognosis including hyperglycemia, advanced glycation end products (AGEP), protein kinase C, and oxidative stress [3]. Recent evidence supporting that inflammation has a critical role in DM complication including DN and it was engaged [4] migration of inflammatory cell to kidney and release of inflammatory cytokines by them such as interleukin IL-1, IL-6, IL-18, and TNF-α, can damage kidney and participate in pathogenesis of DN [5] additionally, a correlation between the high levels of these cytokines in serum and urine with the progression of DN was found [6].

Numerous reports elucidates that chronic hyperglycemia stimulated reactive oxygen species (ROS) production which have a key role in the development of diabetic complications [7]. Reactive oxygen species (ROS) can induce various proinflammatory transcriptional factors, leading to the production of cytokines, adhesion molecules and chemokines, as well an inflow of inflammatory cells into the kidney [8]. SOD activity is related to oxidative damage in patients undergoing from metabolic disorders, therefore it could be used as a biomarker to define the level of oxidative stress in the different diseases like DN [9].

miRNAs are small, non-coding have 21-23 nucleotides, which inhibit gene expression by binding with the 3-untranslated region (UTR) of their respective mRNAs [10]. In normal physiology, noncoding RNAs were considered as major regulators of gene expression and play a central role in the arranging of essential cellular activities like development, proliferation, differentiation, apoptosis, immune regulation [11][12].

Several studies were indicated the main roles of numerous miRNA in maintaining metabolic homeostasis and therefore, regulation of these miRNAs could serve as potential therapeutic in metabolic disorders [13]. miRNA-146a, have anti-inflammatory effect in the pathogenesis of diabetic complication like nephropathy, retinopathy, and cardiovascular complication, and have been considered as potent diagnostic markers of inflammation in these conditions [14].

Overall, this study aimed to investigate the effects of miR-146a in regulation of inflammation response during DN.

MATERIALS AND METHODS

A total of 90 subjects were included in this study, and categorized accordingly into three groups: type 2 diabetes mellitus (DM) patients (n=30, F=17, M=13), diabetic nephropathy (DN) patients (n=30, F=12, M= 18), and healthy controls (n=30, F=15, M=15). The mean age of control (52.9 ± 13.1years), the patient groups (55.33±11.8 years) which were randomly selected from August to November 2017. This study was approved by the Ethics Committee of Clinic Hospital. all subjects were asked to fill a written informed consent.

All patients engaged in study were clinically diagnosed in Al-Diwanyah teaching hospital had type two diabetes mellitus medical history of each patients was acquired, including sex, age, diseases suffered and illness duration. Control group subjects, who were clearly healthy in expression of being non-diabetic, with no other endocrine problem or metabolic renal diseases, acute illness or infection were chosen.

5 ml blood were collected in Vacutainer tubes with clot activator from individual. Serum was isolated through centrifugation at (4000 rpm) (Gottingen, Germany) for 10 minutes. The separated serum was divided into two parts using Eppendorf tubes (0.3 ml), one part kept at (-80 °C) for miRNA analysis, while the other was kept at(-20 °C) for biochemical analysis. HbA1c and serum levels of creatinine, blood urea, albumin, were determined by an automated analyzer (Abbott, USA). Serum superoxide dismutase (SOD) activity is determined spectrophotometry by (Misra and Fridovich.1972) method [15]. Serum level of TNF-α was measured by ELISA, while qPCR was employed to determine serum level expression of 146a miRNA. 200 µl of serum was used for extraction of microRNA using Serum/Plasma microRNA purification kit (Bioworld, USA). The cDNA was prepared from miRNA with Poly Polymerase (A) Tailing by using the miRNA cDNA synthesis kit (abm, Canada). Then, the PCR was conducted using cDNA BrightGreen master mix for miRNA qPCR(Abm, Canada). Forward and reverse universal primers for miRNA were used.

Statistical analysis

Data were analyzed using SPSS software20. Data are expressed as mean± SEM. One-way ANOVA followed by the Tukey *post hoc* analysis or a non-parametric ranking (Kruskal-Wallis) were carried out as appropriate to compare multiple groups for normal and non-normal distribution data respectively. *A* $P < 0.05$ was considered significant throughout.

RESULTS

Patients' characteristics and the biochemical assays data are shown in Table 1.

Compared with the type 2 DM patients and the control, the HbA1c levels were significantly increase in DN ($P < 0.05$). Albumin concentration was decreased significantly in DN group compared to DM and control groups ($P < 0.05$). Also serum SOD activity was significantly decrease in the DN and type 2 DM groups compared to the control. ($P < 0.05$, Fig. 1).

As shown in Fig.2 TNF-α serum levels were significantly increase in DN and type2 DM groups compared to control group (*P* value < 0.05). According to miRNA analysis by qPCR, a significant decrease was indicated in serum levels of miRNA-146a expression in DN group compared to control group ($P < 0.05$, Fig.3), but it levels were none significantly decrease in type 2DM group compared to control group.

miRNA-146a levels were negatively correlated with the levels of Hb1Ac (r = -0.723, $P < 0.05$, Fig.4), but positively correlated with the SOD activity (r = 0.5663, $P < 0.05$, Fig.5) in DN group.

Table 1: Comparison between the data of the patients with DM, DN and control groups.

	Control	DM	DN
Number	30	30	30
Age (years)	52.9 ± 13.1	54.7 ± 12.5	56.6 ± 12.4
BMI (Kg/m2)	26.5 ± 4.7	26.3 ± 5.7	26.0 ± 3.9
FBS (mg/dl)	93.96 ± 5.48	233.03±81.15	244.3 ±61.80*
HbA1c (%)	4.82 ± 0.49	7.13 ± 1.09*	9.72 ± 1.36*
Albumin (mg/dl)	4.29 ± 0.52	4.18 ± 0.42	2.38 ± 0.34*

* $P < 0.05$

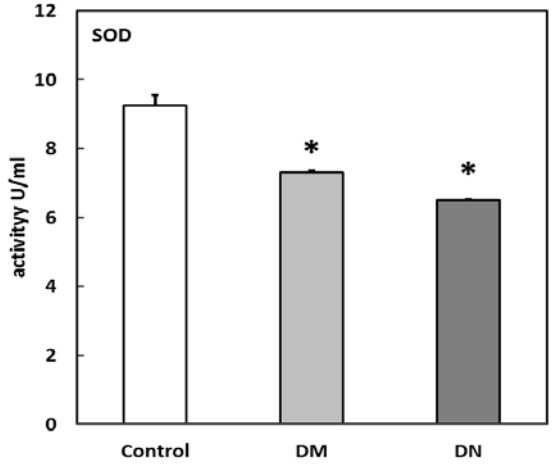


Figure 1: Serum SOD activity in patients Type 2DM and DN. Serum samples were isolated from the blood of patients with DM and DN. SOD activity was measured by colorimetric method. Data are expressed as means ± SEM, for 60 patients, *n* = 60 and 30 control, n=30 with triplicate measurements. *indicates significant differences compared to the control ($P < 0.05$)

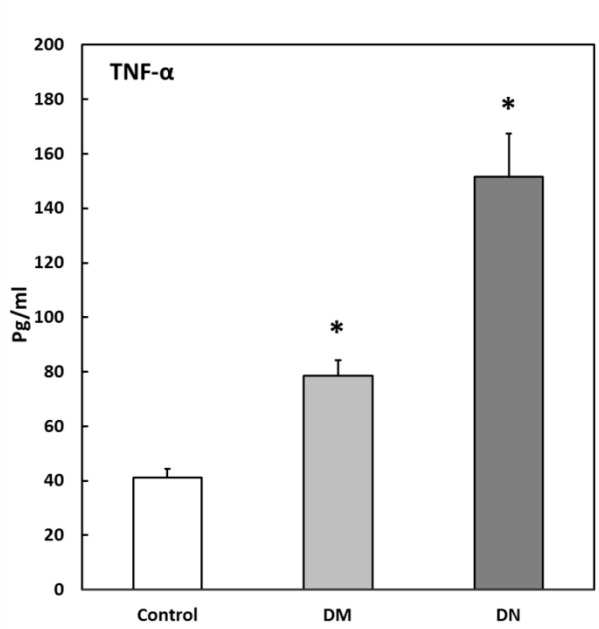
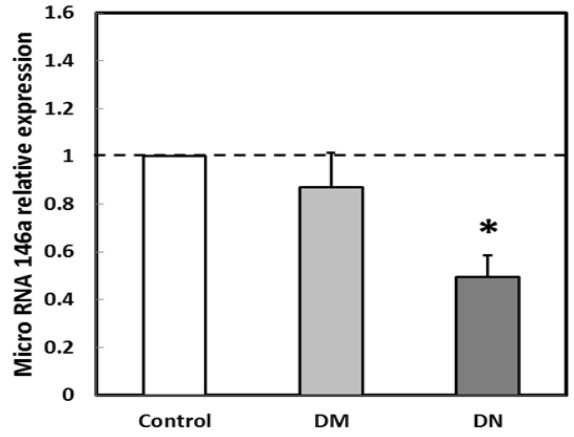


Figure 2: TNF-α production in serum patients with Type 2DM and DN. Serum samples were isolated from the blood of patients with T2DM and DN. TNF-α was measured by ELISA. Data are expressed as means ± SEM, for 60 patients, *n* = 60 and 30 control, n=30. *indicates significant differences compared to the control ($P < 0.05$)



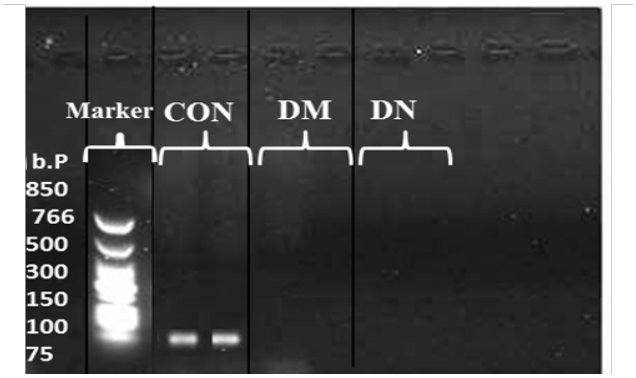


Figure 3: Mic RNA 146a expression in serum patients with Type 2DM and DN. Serum samples were isolated from the blood of patients with T2DM and DN. miRNA 146a was quantified by qPCR. Data are expressed as means ± SEM, for 60 patients, *n* = 60 and 30 control, n=30. *indicates significant differences compared to the control ($P < 0.05$)

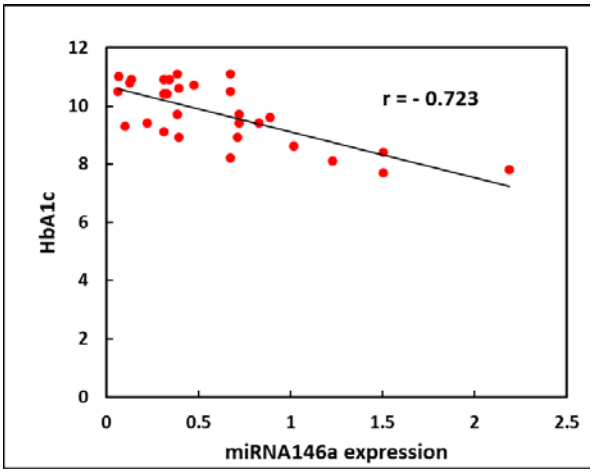


Figure 4: Correlation between miRNA 146a expression and HbA1c in patients with DN.

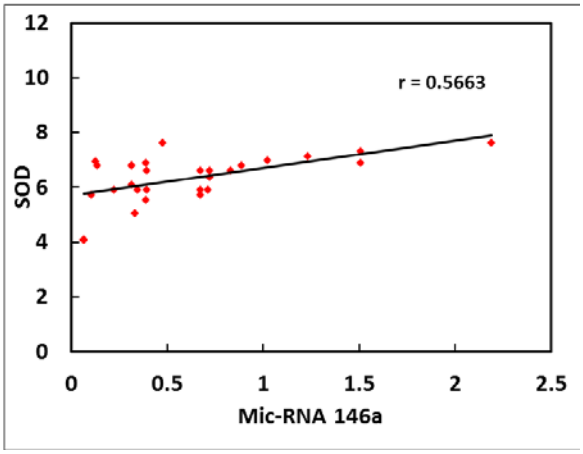


Figure 5: Correlation between miRNA146a and SOD activity in patients with DN

DISCUSSION

The results of present study indicated that miRNA-146a levels decreased with inflammatory response under hyperglycemic condition in DN. Previously reports suggested there is a link between inflammation and development of DN [16].

Immune cells were infiltrated into kidney under hyperglycemic conditions and produced pro-inflammatory cytokines, these affects almost damage renal cells and create inflammatory condition [17]. In this study found a significant increase of HbA1c in DN group compared to type 2 DM and control groups, as a reference in monitoring glycemic control in type 2 DM patients, presence of hemoglobin glycosylation causes a decline of hemoglobin affinity to oxygen [18]. A significant decrease in albumin levels was reported in DN group compared to control. Albumin Excretion was increased with duration of diabetes disease, which was also associated with diabetic nephropathy [19].

Oxidative stress is related with pathogenesis of diabetes and its vascular complications [20]. ROS production in response to chronic hyperglycemia was induced the oxidative stress due to an increase in protein glycation [21]. Studies suggest that hyperglycemia causes oxidative damage in type 2 DM [22]. Moreover, treatment of diabetic rats with antioxidants was shown to afford some protective effects against glucose toxicity [23]. ROS can lead to cells damage by oxidizing proteins, membrane lipids and nucleic acids. However, genes that engaged cell defense and damage may stimulate by ROS [24].

The present results found a decrease in serum SOD activity (Fig. 1) which in agreement with Kumawat M, et al. (2013) results [9], they reported a decrease in SOD activity in DN patient. Considerable reduction in SOD activity may be related to fact that enzymatic proteins were glycated . Nearly a half of percentage of erythrocytes SOD in diabetic patients is glycated, leading to decrease its activity [25].

The mechanisms that involved in the modulation of inflammatory response in DN are still unclear. TNF-α levels was increased significantly in DN group compared to type 2 DM and control groups. TNF-α is a main pro-inflammatory cytokine, and play major roles in the development of diabetic kidney disease [26] through increases ROS formation by numerous ways: First, TNF-α induces ROS generation by downstream events including the mitochondria. Second, TNF-α promotes the transcription of different component of NADPH oxidase enzyme, or enhance its activity [27], leading to stimulate the production of cell adhesion molecules [28], which affect the renal mesangial cells and subsequently the kidney function [29]. On the other hand, TNF-α is induced a reabsorption of sodium solute by epithelial sodium canal activation in proximal tubule cells, that causes sodium preservation and consequently activate TGF-β release, this improved renal hypertrophy throughout the initial stage of DN [30]. The elevated levels of circulatory proinflammatory cytokines are thought due to inflammatory cell (monocytes/macrophages) activation [31] which can accelerate DN progression.

miRNA-146a is involved in the negative feedback mechanism that modulates this inflammatory response [32].Expression of miRNAs, like miR-146a, has been indicated in diabetic complications, including DN [32,33]. A decrease miR-146a expression during DN have been reported in some studies [34], while others have been shown an increase expression [33] the present results show a significant lower miR-146a expression in DN group (Fig. 3).

In the context of diabetic retinopathy and nephropathy, miRNA-146a has a significant role in the specific inflammatory cytokines and extracellular matrix (ECM) proteins release [34]. miRNA-146a plays an important anti-inflammatory and protective role in the pathogenesis of DN and consider as immune-modulatory miRNAs [12]due to regulation of oxidative stress and the production of pro-inflammatory factors [35].

Recent research on diabetic rodents demonstrated that overexpression of miRNA-146a inhibits oxidative stress and decreases the construction of inflammatory factors [36], while reduced miR-146a expression lead to oxidative stress and activate transcription factor NF-κB, resulting augmented inflammatory cytokine and assembly ECM protein affecting pathological alterations in the renal tissues [14]. additionally , it was reported that miR-146a expression was down-regulated in the serum of type 2 DM patients, regarding as a signal of chronic inflammatory state [37] .

A negative correlation between the expression of miRNA-146a and HbA1C (r= - 0.723) while a positive correlation with SOD activity (r= 0.5663) were found (Figs. 4,5), These finding were in agreement with other studies which showed that the family of miRNA-146a engaged in the regulation of oxidative stress associated with antioxidant defense and proinflammatory factors production [35,36].

CONCLUSION

Down expression of miRNA-146a led to increase oxidative stress and consequently decrease antioxidant enzyme defense that may be contributed to stimulation of inflammation, these finding provided an evidence for a protective miRNA-146a as anti-inflammatory modulator of the DN, which may serve as potential biomarker and therapeutic target for DN.

ACKNOWLEDGEMENT

We thank all participants in the study and members of the Al- Diwanyah teaching hospital/clinical chemistry lab.

REFERENCES

1.Tuttle KR, Bakris GL, Bilous RW, et al. Diabetic kidney disease: a report from an ADA Consensus Conference. Am J Kidney Dis 2014; 64:510- 533.

2. Reidy K, Kang HM, Hostetter T, Susztak K. Molecular mechanisms of diabetic kidney disease. J Clin Invest. 2014; 124: 2333–2340.

3- Guo1 J, Li1 J, Zhao J, Yang S, Wang L, Cheng1 G, Liu D, Xiao J, Liu Z & Zhao Z. MiRNA-29c regulates the expression of inflammatory cytokines in diabetic nephropathy by targeting tristetraprolin. Scientific Reports. 201 ; 7: 2314

4- Ni, W. J., Tang, L. Q. & Wei, W. Research progress in signalling pathway in diabetic nephropathy. Diabetes Metab Res Rev. 2015;31: 221–233.

5- Liu, Y. Cellular and molecular mechanisms of renal fibrosis. Nat Rev Nephrol. 2011; 7: 684–696.

6- Maeda, S. Do inflammatory cytokine genes confer susceptibility to diabetic nephropathy? Kidney Int. 2008; 74: 413–415.

7- Wagener FA, Dekker D, Berden JH, Scharstuhl A, van der Vlag J. The role of reactive oxygen species in apoptosis of the diabetic kidney. Apoptosis 2009; 14:1451–1458.

8- Neusser MA, Lindenmeyer MT, Moll AG Human nephrosclerosis triggers a hypoxia-related glomerulopathy. Am J Pathol 2010;176:594–607.

9- Kumawat M, Sharma TK, Singh I, Singh N, Ghalaut VS, Vardey SK, and Shankar V. Antioxidant Enzymes and Lipid Peroxidation in Type 2 Diabetes Mellitus Patients with and without Nephropathy. N Am J Med Sci. 2013; 5(3): 213–219.

10- Kloosterman WP, Plasterk RH. The diverse functions of microRNAs in animal development and disease. Dev Cell. 2006; 11: 441-450.

11- Chandrasekaran, K.; Karolina, D.S.; Sepramaniam, S.; Armugam, A.; Wintour, E.M.; Bertram, J.F.; Jeyaseelan, K. Role of microRNAs in kidney homeostasis and disease. Kidney Int. 2012; 81: 617–627.

12- Bhatt K, Lanting LL, Jia Y, Yadav S, Reddy MA, Magilnick N, Boldin M, Natarajan R. Anti-Inflammatory Role of MicroRNA-146a in the Pathogenesis of Diabetic Nephropathy. J Am Soc Nephrol. 2016; 27(8): 2277–2288.

13-Trajkovski M, Hausser J, Soutschek J, Bhat B, Akin A. MicroRNAs 103 and 107 regulate insulin sensitivity. Nature. 2011; 474: 649-653.

14- Chen, S., Feng, B., Thomas, A. A., and Chakrabarti, S. miR-146a regulates glucose induced upregulation of inflammatory cytokines extracellular matrix proteins in the retina and kidney in diabetes. PLoS One 2017b;12: e0173918.

15- Misra, H.P., and Fridovich, I. The role of Superoxide anion in the auto-oxidation of epinephrine and a simple assay for Superoxide Dismutase. J. Biol. Chem., 1972; 247: 3170-3175.

16- Lim AK, Tesch GH: Inflammation in diabetic nephropathy. MediatorsInflamm 2012: 146154.

17- Tesch GH: Macrophages and diabetic nephropathy. Semin Nephrol. 2010;30: 290–30.

18- Artyukhov VG. Gemoproteidy: zakonomernosti fotokhimicheskikh prevrashcheniy v usloviyakh razlichnogo mikrookruzheniya. Voronezh: Izdatelstvo Voronezhskogo universitetata 1995, 280.

19- Ibrahim S, Bulgurlu S.S., Demirtunc R. The Relation between HbA1c and urine albumin excretion in type2 diabetes mellitus patients. Acta Medica Mediterranea. 2017; 33: 65.

20- Pitocco D, Tesauro M, Alessandro R, Ghirlanda G, Cardillo C. Oxidative stress in diabetes: implications for vascular and other complications. Int J Mol Sci. 2013; 14: 21525-21550.

21- Matsuoka T, Kajimoto Y, Watada H, Kaneto H, Kishimoto M, Umayahara Y, Fujitani Y, Kamada T, Kawamori R, Yamasaki Y: Glycation-dependent, reactive oxygen species-mediated suppression of the insulin gene promoter activity in HIT cells. J Clin Invest. 1997; 99:144–150.

22- Ihara Y, Toyokuni S, Uchida K, Odaka H, Tanaka T, Ikeda H, Hiai H, Seino Y, Yamada Y: Hyperglycemia causes oxidative stress in pancreatic betacells of GK rats, a model of type 2 diabetes. Diabetes. 1999; 48:927–932.

23- Ihara Y, Yamada Y, Toyokuni S, Miyawaki K, Ban N, Adachi T, Kuroe A, Iwakura T, Kubota A, Hiai H, Seino Y. Antioxidant alpha-tocopherol ameliorates glycemic control of GK rats, a model of type 2 diabetes. FEBS Lett.2000; 473:24–26.

24- Nishikawa T, Edelstein D, Du XL, Yamagishi S, Matsumura T, Kaneda Y, Yorek MA, Beebe D, Oates PJ, Hammes HP, Giardino I, Brownlee M. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. Nature.2000; 404:787–790.

25- Arai K, Lizuka S, Tada Y, Oikawa K, Taniguelui N. Increase in the glycosylated form of erythrocyte Cu Zn SOD in diabetes and association of non-enzymatic glycosylation with enzyme activity. Biochim Biophys Acta. 1987;924: 292–6.

26- Sindhughosa DA, Pranamartha GMK . The involvement of proinflammatory cytokines in diabetic nephropathy: Focus on interleukin 1 (Il-1), interleukin 6 (Il-6), and tumor necrosis factor-alpha(TNF-A) signaling mechanism. Bali Med J. 2017; 6 (1): 44-51.

27 - Morgan MJ, Liu ZG. Reactive oxygen species in TNF alpha-induced signaling and cell death. Mol Cell 2010; 30:1–12.

28- Duran-Salgado MB, Rubio-Guerra AF. Diabetic nephropathy and inflammation. World J Diabetes. 2014; 15: 5(3): 393-398

29-Raedke HH, Meier B, Topley N, Fluge J, Habermehl GG, Resch K. Interleukin 1-alpha and tumor necrosis factor-alpha induce oxygen radical production in mesangial cells. Kidney Int. 1990; 37:767-75.

30- Navarro JF, Mora C, Macía M, García J. Inflammatory parameters are independently associated with urinary albumin in type 2 diabetes mellitus. American Journal of Kidney. Diseases. 2003; 42(1): 53–61.

31- O'Connell RM, Rao DS, Baltimore D. microRNA regulation of inflammatory responses. Annu Rev Immunol. 2012; 30: 295– 312.

32- Feng B, Chen S, McArthur K, Wu Y, Sen S, Ding Q, Feldman RD, Chakrabarti S.: miR-146a-Mediated extracellular matrix protein production in chronic diabetes complications. Diabetes. 2011; 60: 2975–2984.

33- Huang Y, Liu Y, Li L, Su B, Yang L, Fan W, Yin Q, Chen L, Cui T, Zhang J, Lu Y, Cheng J, Fu P, Liu F. Involvement of inflammation-related miR-155 and miR-146a in diabetic nephropathy: Implications for glomerular endothelial injury. BMC Nephrol. 2014; 15: 142.

34- Capitão M, Soares R. Angiogenesis and Inflammation Crosstalk in Diabetic Retinopathy. J Cell Biochem. 2016; 117:2443-2453.

35- Fulzele S, El-Sherbini A, Ahmad S et al. MicroRNA-146b-3p regulates retinal inflammation by suppressing adenosine deaminase-2 in diabetes. Biomed Res Int. 2015; 2015: 846501.

36- Wang HJ, Huang YL, Shih YY, Wu HY, Peng CT, Lo WY. MicroRNA-146a decreases high glucose/ thrombin-induced endothelial inflammation by inhibiting NAPDH oxidase 4 expression. Mediat Inflamm. 2014; 2014: 379537.

37- Baldeon RL, Weigelt K, de Wit H et al. Decreased serum level of miR-146a as sign of chronic inflammation in type 2 diabetic patients. PLoS One. 2014; 9: e115209.