The Role of Advanced Glycated End Products and Microalbuminuria on Developing Diabetic Retinopathy in Type 2 Diabetes Mellitus

Thanaa Mohammed Juda[1] Ahmed Mousa Issa[2] Haider Kamil Zaidan[3]
[1] College of Medicine, University of Babylon, Hilla, Iraq.
[2] College of Medicine, University of Kufa, Kufa, Iraq.
[3] College of Science, University of Babylon, Hilla, Iraq.

Received 8 October 2013 **Accepted 31 October 2013**

Abstract

Diabetic retinopathy is a frequent microvascular complication of diabetes mellitus . We analyzed the association of serum level of major advanced glycated end product mainly Nε-carboxymethyl-lysine with prevalence of developing of diabetic retinopathy in Type 2 diabetic patients , a case control study were confirmed on patients with type 2 diabetes mellitus whom were examined by an ophthalmologist at AL- Hilla Teaching Hospital and patients classified into proliferative and non proliferative diabetic retinopathy and the level of Nε-carboxymethyl-lysine were 2.2±0.45 and2.9±0.24 among NPDRP and PDRP respectively and the level among control diabetic patients without retinopathy 1.2±0.2 and the statistical analysis were revealed that were significant difference between patients and control at p value <0.05 Estimation of microalbuminuria among diabetic patients revealed that prevalence of microalbuminuria among patients with NPDRP is (68%) ,and among patients with PDRP are (80%) ,while prevalence among diabetic patients without retinopathy are (33%). The odd ratio for association between microalbuminuria and diabetic retinopathy is calculated and the results is 2.5 which indicate that patients with positive microalbuminuria is at higher risk for developing retinopathy and microalbuminuria is considered risk marker for developing diabetic retinopathy.

Keywords: advanced glycated end products, carboxymethyl-lysine, diabetic retinopathy, proliferative diabetic retinopathy, risk marker, odd ratio.

الخلاصة

اعتلال الشبكية السكري من مضاعفات الأوعية الدموية الدقيقة لداء السكري.تمت دراسة مدى الترابط بين مستوى متراكمات التسكر ا لمتقدمة مثل كاربوكسي مثلايسين مع تطور مرض اعتلال الشبكية السكري .هذه الدراسة تمت في مستشفى الحلة التعليمي لمرضى السكري النوع الثاني تم تشخيص مرض اعتلال الشبكية السكري من قبل أخصائي العيون وتم تصنيف المرض إلى اعتلال الشبكية السكري المتكاثر و غير المتكاثر.كان مستوى كاربوكسي مثلايسين في النوع غير المتكاثر٠,٤٥± ٢,٢وفي النوع المتكاثر ٢,٩±٠,٢٤ وفي مجموعة السيطرة٠,٢ ±١,٢.

p<0.05 عند مستوى التحليل الإحصائي أوضح بان هناك فرق معنوي بين المرضى و مجموعة لسيطرة

بيلة الألبومين الزهيدة تم تقديرها وكانت نسبتها عند مرضى اعتلال الشبكي السكري المتكاثر(٨٠%) و(٦٨%) في النوع غير متكاثر ومستواه عند مجموعة السيطرة(٣٣ %)وكانت نسبة الخطورة بين بيلةالالبومين الزهيدة وتطور اعتلال الشبكية السكري هو (٢,٥) وهذا يؤكد إن بيلة الألبومين الزهيدة تعتبر علامة خطر لتطور مرض اعتلال الشبكية السكري.

Introduction

Diabetic retinopathy (DR), can be defined as damage to microvascular system in the retina due to prolonged hyperglycemia[1]. DR is the most common and specific microvascular complication of diabetes**,** It remains a

major cause of visual impairment worldwide among the people in working age and is a leading cause of visual loss in older patients[2].

DR is broadly classified as either non proliferative diabetic retinopathy (NPDR) or proliferative diabetic retinopathy (PDRP).These depend on microvascular changes in the retina as studied by ophthalmoscopy [3] . Fundus abnormalities in diabetic patients have a progressive course, from mild retinopathy non proliferative, where microaneurisms are the main feature[4,5]. to severe proliferative disease with neovascularizationof the disc, retina and iris[6.7] .

Advanced glycation end products (AGEs) have been implicated as causal factors in the complications of diabetes mellitus[8]. The term "AGEs" refers to post translationally glycated modifications on end- standing aminogroups on proteins, lipoproteins, lipids and nucleic acids that non-enzymatically have undergone irreversible dehydration and condensation processes via various reactive intermediates. [9]. The modification itself is often referred to as an "adduct". There are several alternative routes into forming AGEs, and the predominant substrate fuelling the glycation is glucose Possible pathway for formation AGEP are showed in figure no.1[10].

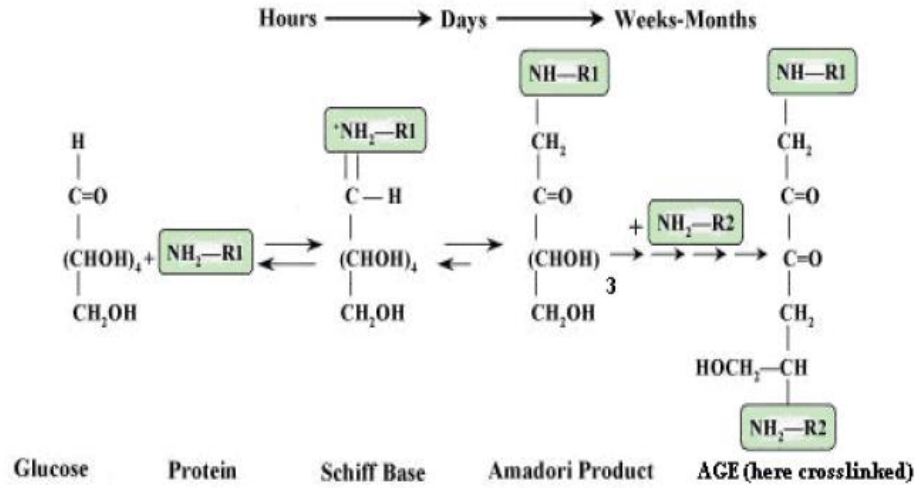

Figure 1 pathway for formation AGEP.

One of important AGEs is $N_{epsilon}$-(carboxymethyl)lysine (CML) which formed from(glyc*oxid*ation of AGEs [11]. AGEs believed to be of pathogenica importance in microvascular complications developing[12].

Microalbuminuria is now defined as a urine albumin excretion between 20 and 200 µg/min (or 15 to 150 µg/min overnightor [13]. However, these values are currently detectable by semi-quantitative dipstick tests, and can be accurately measured by several widely available sensitive methods such as ELISA, RIA, and nephelometry[[14].

Microalbuminuria is often present at the time of diagnosis either due to insidious nature and asymptomatic initial years of type 2 diabetes,or its positive association with insulin resistance[15] The importance of microalbuminuria as an independent predictor of progressive renal disease and cardiovascular

mortality was thereafter realized in a number of prospective and epidemiological studies particularly in patients with diabetes and hypertension[16,17] The prevalence of microalbuminuria among diabetic patients is (15-20-%).

Microalbuminuria in diabetic patients is a risk marker not only for kidney and cardiac disorders but also for severe Persistence ocular morbidity[18].

Material and Method

A case control study was done on 125 patients with NPDRP and PDRP as well as control groups

The subjects participated in this study were classified into following:-

Group one: 75 patients with 2 DM– 75 (46 males and 29 females) with retinopathy ranging from NPDR to proliferative retinopathy. Non proliferative ranging into mild, moderate and severe diabetic retinopathy

Group two: patient with type 2 diabetes mellitus without retinopathy

Group three: apparently healthy subjects were chosen as healthy controls, they were non smoker, non alcohols, and did not have any history of chronic diseases.

Exclusion criteria

1-Congestive heart failure
2-Urinary tract infection
3-Fever
4-Perepheral neuropathy
6-Renal disease
7 - Patients with type 1 diabetes mellitus
8- Gestational diabetes

Methods

1. OxiSelect™ Advanced Glycation End Product (AGE) Kit for determination CML in serum by ELISA [19] . The standard curve for estimation CML are shown in figure 2

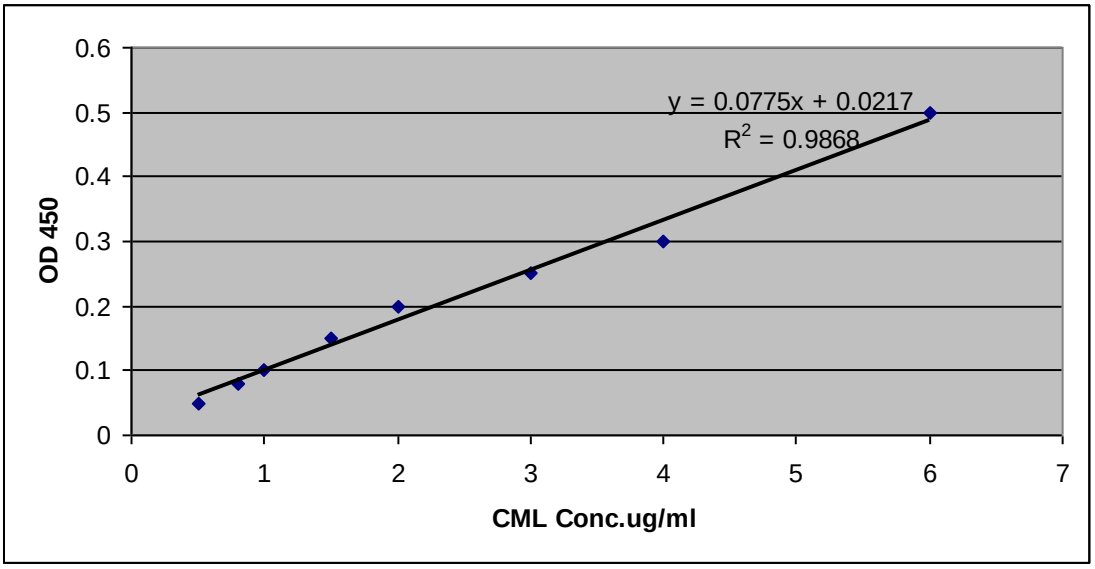

Figure2: Standard curve of CML

2. **Microalbuminurea** determined Strips for the, semi- quantitative in vitro determination of urinary albumin up to a concentration of 100 mg/L.

Principle

Immunological detection of human albumin by means of soluble antibody gold-conjugate. Excess conjugate is retained in a separation zone containing immobilised human albumin immunological[20].

Statistical analysis were performed using SPSS17:0(SPSS Inc, Chicago ,11,USA) The odds ratio

(Ors) and 95% confidence intervals (CIs) for possible risk factors in diabetic retinopathy were calculated .Statistical significance was defined as $P < 0.05$

Results

1.Descriptive study for DRP patients groups

The demographic and medical characteristics of patients are shown in table 1.

Table 1 Patients clinical characteristics

Characteristics	DRP		No DRP	P Value
Age (Years)	NPR 56±8		55.5± 4.5	0.8
	PR 51± 9.4			
Gender	M 46 F 29		M 16 F 9	0.3
BMI(KG/M^2)	NPR	M 27±4.2 F 27±4.2	M 30±2.1 F 24±2.5	0.5
	PR	M 30±4. F 24±**2.**		
HbA1c%	NPR 8.2±o.8		7.6± 0.6	0.005*
	PR 10.2±2.4			
Duration in years	NPR 17±**6**		5.5	0.001*
	PR 12±**6.2**			

2. Microalbumiuria and DRP

Microalbuminuria was estimated in diabetic retinopathy and control group and the relationship between positive microalbuminuria and developing of DRP are represented in figure 3

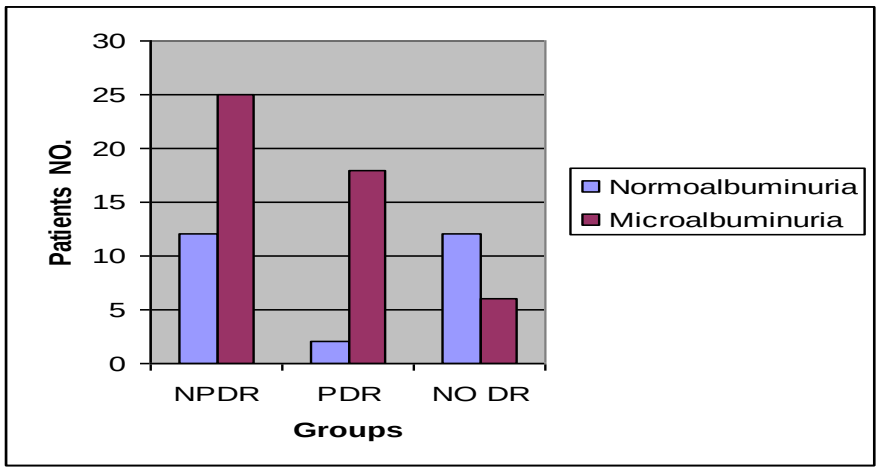

Figure 3 Relationship between microalbuminuria and DRP

3. The relationship between gender and microalbuminuria: The relationship between gender and developing of diabetic retinopathy were represented in figure 4

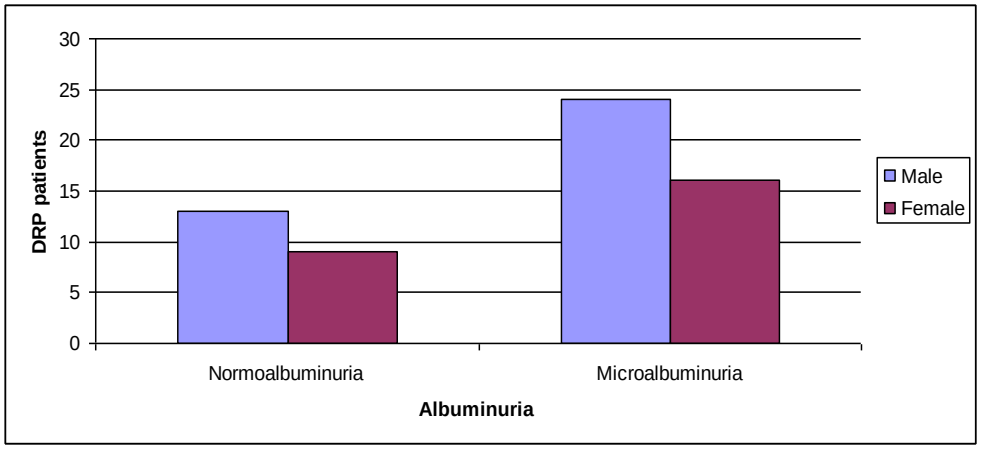

Figure 4 The relationship between gender and microalbuminuria.

3. The relationship between duration of DRP and microalbuminuria: The association of microalbuminuria with duration are representing in figure 5

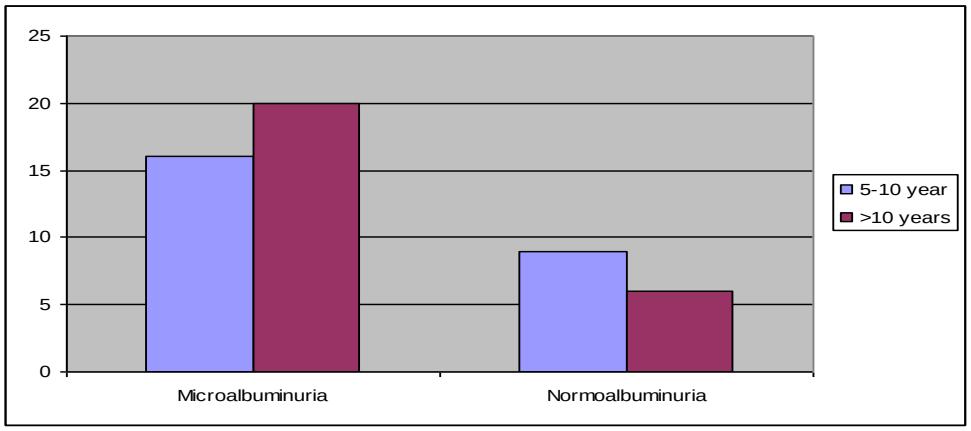

Figure 5: The relationship between duration of DRP and microalbuminuria .

4. DRP and CML

$N^{epsilon}$ CML is belonged to one of advanced glycated end products was measured by ELISA technique and mean± SD are summarized in table1

Table 2 Mean and standard deviation of CML in diabetic retinopathy and control.

GROUPS	CML Mean ± SD (Ug/ml)
Mild DRP	1.8±0.3
Moderate DRP	2.2±0.45
Severe DRP	2.6±0.2
Proliferative DRP	2.9±0.24
Control DNR	1.2±0.2
Control HC	0.6±0.05

The comparism of CML level between diabetic retinopathy (proliferative and non proliferative) with control group are represented in figure 6.

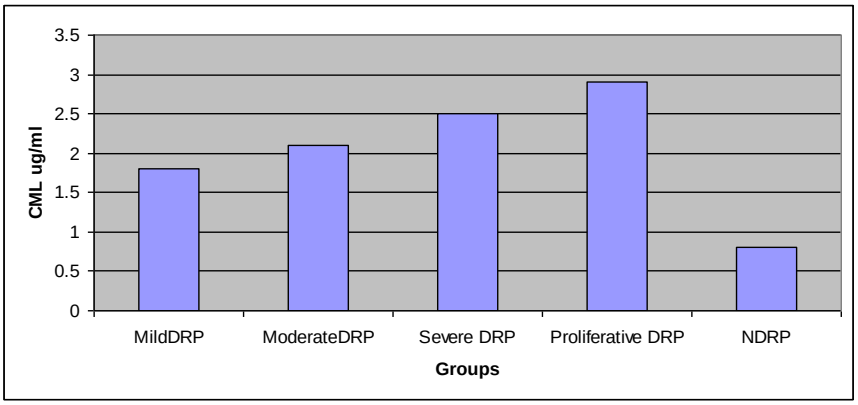

Figure 6 The comparism of Nε-carboxy(methyl)lysine (CML)in sera of type 2 diabetic patients with and without retinopathy.

The ANOVAs analysis for comparism mean among different groups for patients with diabetic retinopathy and control group are represented in following table

Table 3 A nova analysis for comparism CML between control and DRP patients.

CMLug/mL		
Comparism group	**Diabetic retinopathy**	**P value**
Control DM NDRP	**MildNDRP**	**o.oo1***
	Mod.NDRP	**0.001***
	Severe NDRP	**0.001***
	PDRP	**0.001***

(P-value of < 0.05* was considered to be statistically significant)

Advanced glycated end product as CML are considered risk factor for developing diabetic microvascular complication as diabetic retinopathy and The associated between CML with chronic hyperglycemia among diabetic retinopathy patients were represented by Linear regression in figure 7 .

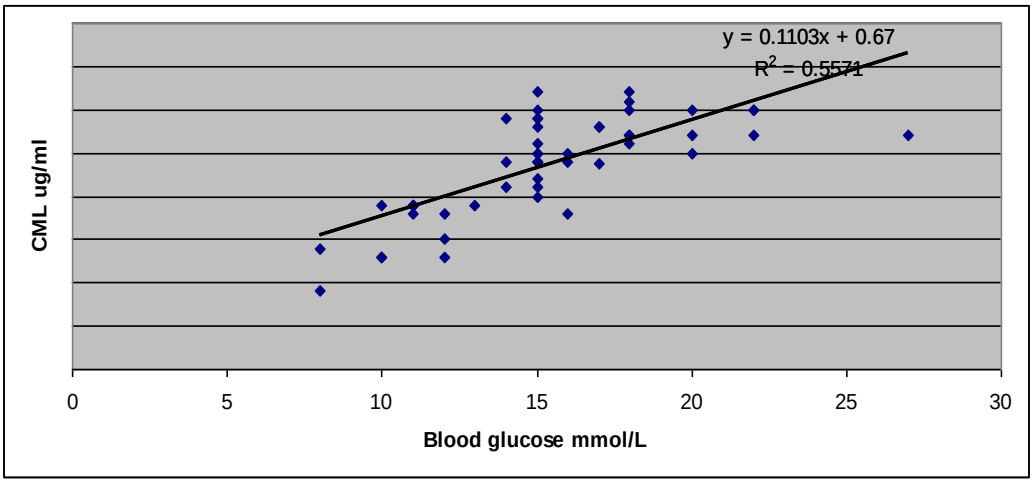

Figure 7 linear regression of CML and blood Glucose .

Table 4 The odd ratio for independent variables as possible risk factor for DRP .

Independent variable	Odd ratio	95%CI	P-Value
DURATION >5	**5.3**	1.9-13.4	0.001*
INSULIN R AND DRP	4.5	1.6-9.4	0.001*
FAMILY HISTORY	3.2	1.3-6.4	<0.01*
Hyperglycemia and DRP	2.6	1.44.3	<0.001*
Microalbuminuria and DRP	2.5	1.4	<0.001*
Obesity and DRP	1	0.7-0.9	0.3

* significant DRP diabetic retinopathy ,CI confidence interval

Discussion

The age of patients varied from 43 to 68 years old (average 56±8±51± 9.4years for NPDRP and PDRP). Patients 'profile data such as sex, family history and type of treatments are shown in Tables 1.

In figure 3 were revealed that microalbuminuria is associated with developing and progression of diabetic retinopathy ,the prevalence of microalbuminuria among patients with non proliferative DRP is 68% ,and among patients with proliferative DRP are 80% ,while prevalence among diabetic patients without retinopathy are 33%.

The data in figure 4 were shown the developing of microalbuminuria among males is more prevalent than females, the microalbuminuria is 60% among males and (40%)among female in patients with diabetic retinopathy.

The microalbuminuria in patients with diabetic retinopathy were increased with increasing in duration of diabetes mellitus ,meaning that microalbuminuria and duration represented risk factors for developing diabetic retinopathy .

Microalbuminuria is considered as marker and predictor for developing diabetic retinopathy, by calculation of odd ratio for microalbuminuria and developing diabetic retinopathy the odd ratio is 2.5 and the odd ratio for increase duration of DM and developing diabetic retinopathy is 5.3 which mean that microalbuminuria and duration are considered risk factors for developing diabetic retinopathy. Microalbuminuria considered as biomarker for detection of microvascular complication in diabetes mellitus as diabetic retinopathy most resent studies agree with this result[21,22] . and some other studies may opposite this result [23,24].

We performed this a case-control study and had been analyzed the association of CML serum levels with advanced stages of diabetic retinopathy The higher the serum of CML level the higher the likelihood for advanced stages diabetic retinopathy. Therefore CML levels provided a novel progressive risk marker for developing and progression of diabetic retinopathy .

A studies were indicated that there were a direct link of advanced glycation end products, including the late oxidative product Nε-carboxymethyl-lysine with diabetic microvascular complications so CML has a role in developing microvascular complication in diabetic patients that lead to difference Vascular dysfunction, including basement membrane thickening, increased vascular permeability and prothrombotic state, and decreased blood flow and it is a ubiquitous trait of microvascular disease of the retina, nephron, and peripheral nerve [25.26]. Since CML can engage receptors of signal transduction for AGE (RAGE), therefore CML can directly activate key cell signalling pathways and modulate gene expression. Most importantly RAGE expression has been found in the retina, mesangial compartment concomitant with AGE/CML accumulation and may therefore provide a direct link of CML levels and diabetic complications[.27]. Our study suggests that factors influencing levels of protein and lipid glycation and oxidation leading to increased level of late oxidative product of CML and this are of considerable importance in microvascular complications. Factors determining differential CML levels could have a major clinical impact and may lead to a wide range of pathologies including vascular complications[28]. The relation ship between CML and developing and progression of diabetic retinopathy were confirmed by other studies [29,30].

References

1. Paul M. Titchenell and David A. Antonetti .Using the Past to Inform the Future: Anti-VEGF Therapy as a Road Map to Develop Novel Therapies for Diabetic Retinopathy . Diabetes June 2013;62:1808-1815.

2. Marshall M. , Flyvbjerg. .. Prevention and early detection of vascular complications of diabetes. British Med. J., 2006;333,475-480.

3. Early Treatment Diabetic Retinopathy Study (ETDRS) Research Group. Grading diabetic retinopathy from stereoscopic color fundus photographs. Ophthalmology ,1991;98 (Suppl.): 786-806.

4. Wilkinson C.P. Achieving consensus on an international clinical classification for diabetic retinopathy. Program and abstracts of the American Academy of Ophthalmology .Annual Meeting, 2002; 20–23 .

5. Agarwal S ., Raman R ., Kumari R., et al. Diabetic retinopathy in type II diabetics detected by targeted screening versus newly diagnosed in

general practice: Ann Acad Med Singapore. 2006; 35(8):531-5.

6 . Antonia, M., Joussena, B., Neil S., Carien, N. Diabetic Retinopathy. Dev. Ophthalmol ,2007 ;39: 1-12 .

7. .O'Doherty M, Dooley I, Hickey-Dwyer M. Interventions for diabetic macular oedema: a systematic review of the literature. Br J Opthalmol. 2008;92:1581-1590.

8 . Ahmed N ., Thornalley PJ. Advanced glycation endproducts: what is their relevance to diabetic complications. Diabetes Obes Metab ., 2007;9(3):233-45.

9. Padayatti PS., Ng AS, Uchida K ., Glomb MA ., Nagaraj RH. Argpyrimidine, a bluefluorophore in human lens proteins: high levels in brunescent cataractous lenses.
Invest Ophthalmol Vis Sci ., 2001;42(6):1299-304.

10. Nawale RB . ,Mourya VK .Non-enzamatic glycation of protein and complication of diabetes .
J.of Biochemestery and Biophysics ,2006; 45 : pp 337-344 .

11. Coughlan MT., Thallas-Bonke V., Pete J., Long DM, Gasser A., Tong DC ., Arnstein M ., Thorpe SR ., Cooper ME ., Forbes JM. Combination therapy with the advanced glycation end product cross-link breaker, alagebrium, and angiotensin converting enzyme inhibitors in diabetes: synergy or redundancy. Endocrinology., 2007;148(2):886-95.

12. Valantin R, Daniela L, Maria M and Bogdana V. Advanced glycation end products in diabetes mellitus: mechanismof action anfocusedtreatment Proc. Rom. Acad., Series B, 2012: 1:p. 9–19 .

13 . Klaus K .,, Henrik S ., Gorm J ., and Jan Skov Jensen New Definition of Microalbuminuria in Hypertensive Subjects: Association With Incident Coronary Heart Disease and Death. Hypertension. 2005;46:33-37,

14. Lajos M. Measurment of albuminuria with size exclusion chromatography characterization and new perspectives. PhD thesis. University of pecs, Faculty of Medicine 2nd,Department of medicine and nephrological center,2011;p 3.

15. Incerti J ., Zelmanovitz T ., Camargo JL ., et al. Evaluation of tests for microalbuminuria screening in patients with diabetes. Nephrol Dial Transplant ,2005;20:2402-2407.

16. Weir MR . Microalbuminuria in type 2 diabetes: An important,
overlooked cardiovascular risk factor. J Clin Hypertens .,2004; 6: 134–143 .

17. Asselbergs FW ., Diercks GF ., Hillege HL ., van Boven AJ ., Janssen WM. The Prevention of Renaland Vascular Endstage Disease Intervention Trial (PREVEND IT) Investigators: Effects of fosinopril
and pravastatin on cardiovascular events in subjects with microalbuminuria. Circulation . ,2004;110:2809 – 2816.

18. Adler AI ., Stevens RJ., Manley SE ., Bilous RW ., Cull C. Development and progression of nephropathy in type 2 diabetes: the United Kingdom Prospective Diabetes Study (UKPDS 64). Kidney Int .,2003;63:225-32.

19 . Ellisa kit from Cell biolab .

20 . Microalbumiuria kit ,MICRAL-TEST ,An Accu-Chek product.

21..VK Ajoy M, Suneetha N, Jyothi I . Microalbuminuria and low hemoglobin as risk factors for the occurrence and increasing severity of diabetic retinopathy Indian J .of Ophthalmology, 2011 ; 59(3) : 207-210 .

22. Yu-Hsin C, Harn-S, Der-Cherng T .More Impact of Microalbuminuria on Retinopathy Than Moderately Reduced GFR Among Type 2 Diabetic Patients .Diabetes Care ,2012; 35 (4) : 803-808

23. Cruickshanks KJ, Ritter LL, Klein R, Moss SE. The association of

microalbuminuria with diabetic retinopathy. The Wisconsin Epidemiologic Study of Diabetic Retinopathy. Ophthalmology 1993; 100: 862-7

24. Savage S, Estacio RO, Jeffers B, Schrier RW. Urinary albumin excretion as a predictor of diabetic retinopathy, neuropathy, and cardiovascular disease in NIDDM. Diabetes Care 1996; 19: 1243-8.

25. Su-Yen G and Mark E . The Role of Advanced Glycation End Products inProgression and Complications of Diabetes .J Clin Endocrinol Metab, 2008; 93(4):1143–1152 .

26. Meerwaldt R, Zeebregts CJ, Navis G, Hillebrands JL, Lefrandt JD .Accumulation of advanced glycation end products and chronic complications in ESRD treated by dialysis. Am J Kidney Dis 2009;53:138–50

27. .Fosmark DS, Torjesen PA, Kilhovd BK, Berg TJ, Sandvik L, Hanssen KF, Agardh CD, Agardh E: Increased serum levels of the specific advanced glycation end product methylglyoxal-derived hydroimidazolone are associated with retinopathy in patients with type 2 diabetes mellitus. Metabolism ,2006 ;55:232–236 .

28 . Alison Goldin, BA; Joshua A. Beckman, MDAnn Marie Schmidt, MD Advanced Glycation End Products; Sparking the Development of Diabetic Vascular Injury . Circulation. 2006; 114: 597-605

-29. .Nawale R B , Mourye V K .N-enzymatic glycated protein A case complication in diabetes . Indian J .of Biochemestery and Biophysics ,2006 ; 43:pp 332-344 .

30 . Genuth S, Sun W, Cleary P, Sell DR, Dahms W, Malone J, et al. Glycation and carboxymethyllysine levels in skin collagen predict the risk of future 10-yearprogression of diabetic retinopathy and nephropathy in the diabetes control and complications trial and epidemiology of diabetes interventions and complications participants with type 1 diabetes. Diabetes 2005 Nov; 54 (11): 3103-11.