Effects of trace elements on albumin and lipoprotein glycation in diabetic retinopathy

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ABSTRACT

الأهداف: اختبار تأثير بعض العناصر النادرة على كل من تسكّر البروتينات والشحوم البروتينية وشدّة اعتلال الشبكية السكري.

الطريقة: أجريت الدراسة في مستشفى ابن الهيثم التخصصي لأمراض العيون خلال الفترة من فبراير حتى ديسمبر 2008 على 42 مصاب بمر ض السكري 14 لايعانون من اعتلال الشبكيّة، 14 مصاب باعتلال الشبكيّة غير المتكاثر(NPDR) و 14 ممن يعانون من اعتلال الشبكيّة المتكاثر(PDR) . إضافة الى20 من الأصحاء مطابقين المعدل الصحي (NC). تم قياس تسكّر الزلال، الشحوم البروتينية نوع ألفا، بيتا، والسابق لبيتا بطريقة الترحيل الكهربائي باستعمال هلام الاكاروز. وقيست تراكيز عناصر الكادميوم (Cd) و السيلينيوم (Se) و الكروم (Cr) و الخارصين (Zn) والنحاس (Cu) بجهاز امتصاص الطيف الذري عديم الشعلة.

النتائج: وجد ارتفاع معنوي في معدل تسكّر بروتينات الشحوم نوع بيتا في مصل مرضى السكري الغير مصابين باعتلال الشبكيّة مع زيادة بيّنة وغير معنوية (p=0.06) في معدلات كل من تسكّر الزلال وتسكّر بروتينات الشحوم نوع السابق لبيتا لدى مجموعتي المصابين باعتلال الشبكيّة المتكاثر والغير متكاثر على التوالي. علاوة على ذلك، فقد سجّل انخفاض معنوي في معدلات الكروم (p<0.05) ونسبة الخارصين إلى النحاس (p<0.001) عند كافة المصابين باعتلال الشبكيّة السكري. كما وجد أن مستوى الكادميوم يرتفع بازدياد مدة الإصابة بمرض السكري (p<0.001) وبارتفاع مستوى السكر في الدم (p<0.025)، ولكنّ قيم الكروم في المصل تتناقص مع تقدم الإصابة بمرض السكري (p<0.025).

خاتمة: تشترك عمليّتي التسكّر والأكسدة في تطور اعتلال الشبكيّة السكري وأن التغيّرات في تركيز الكادميوم و السيلينيوم و الكروم و الخارصين والنحاس لها بعض التأثيرات على هاتين العمليتين.

Objectives: To test the effect of some trace elements, on protein and lipoprotein glycosylation and their impact on the severity of diabetic retinopathy.

Methods: A case control study was conducted in 42 diabetic patients (14 without retinopathy [DC]; 14 with non-proliferative diabetic retinopathy [NPDR]; 14 with proliferative diabetic retinopathy [PDR]) at Ebin Al-Haitham Specialized Hospital, Baghdad, Iraq for Ocular Diseases from February to December 2008. In addition to 20 age and gender matched healthy controls (NC). The glycation of albumin, alpha-, pre beta-, and beta-lipoproteins was measured by agarose gel electrophoresis. Serum levels of cadmium (Cd), selenium (Se), chromium (Cr), zinc (Zn), and copper (Cu) were analyzed by flameless atomic absorption spectrophotometer.

Results: There was significant elevation in the mean serum glycated beta-lipoprotein in DC (p<0.05) and a near significant increase (p=0.06) in the means of both glycated albumin and pre beta-lipoproteins among the PDR and NPDR groups. Moreover, a significant reductions in serum means of Cd (p<0.05) and Zn/Cu ratios (p<0.001) were recorded in all diabetic retinopaths as compared to DC. The Cd level rises with the increase in duration of diabetes (p<0.001) and hyperglycemia (p<0.025) whereas, the serum Cr values decreases with the progression of diabetes (p<0.025).

Conclusion: Both glycation and oxidative processes are involved in the development of diabetic retinopathy, and changes in the concentration of Cd, Se, Cr, Zn, and Cu have some impact on the disease progression.

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Diabetic retinopathy is a highly specific vascular complication of both type 1 and type 2 diabetes, and is the most common cause of blindness in middle-aged subjects, accounting for at least 12,000 new cases in the United States each year. The clinical signs of diabetic retinopathy are present in nearly all persons who have had type 1 diabetes for 20 years, and in nearly 80% of those with type 2 diabetes of the same duration.[1] The development of diabetic retinopathy is a multifactorial process where genetic, metabolic, and growth factors play an important role. Chronic hyperglycemia leads to oxidative injury, micro thrombi formation, cell adhesion molecule activation, leukostasis, and cytokine activation.[2] Modified (oxidized and/or glycated) low-density lipoproteins have been implicated in retinal pericyte loss, one of the major pathologic features of early-stage diabetic retinopathy. The highly oxidized glycated low density lipoprotein (HOG-LDL) elicits gene expression in retinal pericytes that may contribute to pericyte loss and other retinal abnormalities in diabetic retinopathy.[3] Glucose reacts non-enzymatically with N-terminal and lysyl side chain amino groups in proteins to form fructosamines, which are early stage glycation products. Fructosamines degrade oxidatively and non-oxidatively to form advanced glycation end products. Like other serum proteins, lipoproteins are non-enzymatically glycated in the presence of glucose. The Apo lipoprotein B100 (Apo-B100) of LDL, usually has some of its lysine residues modified by non-enzymatic glycation.[4] The metabolism of metals such as zinc (Zn), copper (Cu), cadmium (Cd), and mercury is regulated by cysteine-rich, low molecular weight intracellular proteins metallothioneins (MT). The lowered MT synthesis may consequently contribute to the increased peroxidation reactions in the affected retinas.[5] Moreover, in a large cross-sectional study conducted on 8,722 adults of ≥40 years of age during the period from 1988-1994 the 24 hour urinary Cd levels were found to be significantly and dose-dependently associated with both impaired fasting glucose and diabetes, and raises the possibility that Cd may cause prediabetes and diabetes in humans.[6] In this study, we aimed to evaluate the effect of some trace elements such as Se, Cr, Cd, Zn, and Cu on the glycation of albumin and lipoproteins and to test their impact on the severity of diabetic retinopathy.

Methods. The study comprised 42 patients (females 18; males 24) with type 2 diabetes mellitus (DM) who attended the Ebin Al-Haitham Specialized Hospital, Baghdad, Iraq of Ocular Diseases from February 2008 to December 2008. A comprehensive medical examination was performed in all subjects, and a medical history check was also made to establish the presence of any complications. A total of 20 age- and gender- matched healthy subjects (females 9; males 11) were also enrolled as normal controls (NC). The following inclusion criteria were adopted for the study participants: non-smokers, not on antioxidant vitamin supplements or minerals, lipid-lowering drugs, beta-blockers, or non-steroidal anti-inflammatory drugs, and no alcohol consumption. Those with renal or liver dysfunction, gastrointestinal disorders, such as malabsorption were excluded from the study. The research was approved by the Local Ethical Committee, Al-Nahrain College of Medicine, Al-Kadymia, Baghdad, Iraq and informed consent was obtained from all patients.

Ophthalmologic examination including visual acuity, intraocular pressure, fundoscopy (utilizing slit lamp and contact lenses), and indirect ophthalmoscopy were carried out. Grading was performed by the senior ophthalmologist and the patients were categorized according to the degree of their retinopathy into 3 groups: no retinopathy (diabetic control [DC], n=14), non-proliferative diabetic retinopathy (NPDR) (n=14), and proliferative diabetic retinopathy (PDR) (n=14).

Retinal lesions were compared with the standard 7-field stereo photograph protocol. The worst eye determines each patient's level of retinopathy. Non proliferative retinopathy (mild to severe) was defined as the presence of one or more micro-aneurysms, hemorrhages, venous beading, and intraretinal microvascular abnormalities. Proliferative retinopathy was defined as any definite neovascularization, pre retinal hemorrhage, and vitreous hemorrhage.[7]

Depending on the duration of diabetes, patients with retinopathy were stratified into 3 subgroups: G1: encompass those who had DM for up to 8 years. G2: include patients who suffered from diabetes for more than 8 years but less than 12 years. G3: involves diabetic retinopaths with a history of DM that exceeds 12 years duration. Blood samples were drawn after a fast of at least 12 hours for the measurement of lipid profile. All analyses were performed according to standard procedures[8] including serum fasting glucose, creatinine, total proteins, and albumin. Serum fructosamine concentration was determined using the fructosamine kit (BioMericks, Hamburg, Germany). Whereas serum concentrations of Zn, Cu, selenium (Se), chromium (Cr), and Cd were measured by flameless atomic absorption[9] spectrophotometry (Shimatzu, Tokyo, Japan).

The concentration of the glycated albumin and lipoproteins was measured as previously mentioned.[10] In brief summary, 1.5 µl of patient and control sera were subjected to agarose gel electrophoresis at 95 V for 45 minutes in a barbital buffer (pH=8.6, µ=0.06). The gel film was then layered with a few milliliters of

7mM nitro blue tetrazolium solution in 0.1M sodium carbonate buffer (pH=10.3) for 3-5minutes.The agarose gel films were overlaid with cellulose acetate membrane that had been soaked with the same buffered stain and incubated at 37°C for 24 hours in the dark. The resulted violet colored bands (**Figure 1**) were eluted with an equal volume of chloroform and the color intensity was measured spectrophotometrically at 545 nm. Each concentration (µmol/L) was calculated from the percentage multiplied by the value of serum fructosamine.

The statistical analysis was carried out using Microsoft ®Excel 2007. The mean, standard deviation, and Student t-test were used for the descriptive statistics and to compare between different groups. A simple correlation coefficient (r) was used to evaluate the relationship of glycated albumin and glycated lipoproteins with all of the serum variables and controlling for the duration of diabetes. A p value of less than 0.05 was considered statistically significant.

Results. Demographic characteristics of the studied subjects and some biochemical test results are listed in **Table 1**. **Table 2** shows the mean concentrations of serum glycated albumin, glycated lipoproteins and some trace elements in normal controls and diabetics with and without retinopathy. There is a statistically significant elevation in the mean serum glycated ß-lipoprotein in

Table 1 - Demographic characteristics and some biochemical data of the study subjects.

Demographic characteristics	Normal control n=20	Diabetic control n=14	Non proliferative diabetic retinopathy n=14	Proliferative diabetic retinopathy n=14
Gender (number)				
Females	9	5	5	8
Males	11	9	9	6
Age (years)	47.8 ± 12.76	53.64 ± 9.278	56.65 ± 8.576	57.22 ± 4.4
Duration of diabetes (years)	-	9.25 ± 6.43	12.49 ± 7.01	8.43 ± 4.35
Serum fructosamine (µM/l)	256.5 ± 28.1	418.0 ± 167.4[a†]	466.9 ± 159.9[a‡]	474.7 ± 149.9[a‡]
Fasting serum glucose (mM/l)	4.38 ± 0.832	7.21 ± 2.544[a†]	10.64 ± 4.813[a‡,b*]	10.72 ± 5.936[a‡]
Serum triglycerides (mM)	1.19 ± 0.505	1.51 ± 0.411	1.58 ± 0.849	2.17 ± 1.419[a*]
Serum total cholesterol (mM/l)	4.37 ± 0.816	5.19 ± 0.67	5.31 ± 1.153	5.21 ± 1.269[a*]
Serum LDL-C (mM/l)	2.62 ± 0.561	3.48 ± 0.65[a‡]	3.3 ± 1.427	3.09 ± 1.358
Serum HDL-C (mM)	1.09 ± 0.215	1 ± 0.18	1.15 ± 0.183	0.92 ± 0.174[a†]
Serum VLDL-C (mM/l)	0.55 ± 0.233	0.65 ± 0.907	0.86 ± 0.666[b*]	1.03 ± 0.623[a*]
Serum LDLC/HDL-C ratio	2.43 ± 0.661	3.62 ± 0.907[a‡]	2.57 ± 1.186	3.95 ± 1.931[a†]
Serum creatinine (µM/l)	69 ± 27.741	83.98 ± 52.258	64.54 ± 34.751	58.73 ± 18.062
Serum total protein (g/dl)	7.12 ± 0.481	7.37 ± 0.892	7.23 ± 0.667	6.97 ± 0.779
Serum albumin (g/dl)	4.43 ± 0.238	4.4 ± 1.476	4.48 ± 0.799	4.22 ± 0.339[a*]
Albumin/globulin ratio	1.68 ± 0.26	1.54 ± 0.28	1.65 ± 0.788	1.5 ± 0.3

Results are expressed as mean ± standard deviation. [a]student t-test: different groups versus normal controls, *p<0.05, †p<0.01, ‡p<0.001
[b]student t-test: different groups versus diabetic controls, *p<0.05.

Table 2 - The mean concentrations of serum glycated albumin, glycated lipoproteins and trace elements in normal controls and diabetics with and without retinopathy.

Groups	Glycated albumin (µM/l)	Glycated ß-lipoprotein (µM/l)	Glycated pre-ß Lipoprotein (µM/l)	Glycated ß-lipoprotein (µM/l)	Selenium (µM/l)	Chromium (nM/l)	Cadmium (nM/l)	Zinc/Cu ratio
Normal control (n=20)	33.38±5.298	22.07±4.896	21.94±4.59	23.75±4.028	29.12±38.865	121.92±50.356	0.35±0.596	0.72±0.208
Diabetic control (n=14)	30.57±4.816	21.49±2.358	22.73±3.791	27.15[a*]±4.306	23.65 ± 21.106	100.8±36.058	0.58[a*]±0.897	1.31[a†]±0.181
Diabetic retinopathy (n=28)	33.32±5.638	20.29 ± 4.985	22.92 ± 5.891	24.59 ± 6.965	28.17 ± 45.289	88.12[a*]±43.395	0.30[b*]±0.525	0.76[b†]±0.212
Non- proliferative (n=14)	32.57±5.472	20.85±4.133	23.96±6.967	24.89±6.563	32.03±47.956	77.49[a†]±41.762	0.43[c*]±0.68	0.75[b†]±0.201
Proliferative (n=14)	34.06±5.508	19.76±5.308	21.88±4.326	24.29±7.333	24.78 [c†]±41.942	98.75±42.382	0.18 [a†, b*]±0.237	0.79[b†]±0.246

Results are expressed as mean ± standard deviation. [a]Student t-test: different groups versus normal controls, *p<0.05, †p<0.001.
[b]Student t-test: different groups versus diabetic controls, *p<0.05, †p<0.001.
[c]Student t-test: non proliferative diabetic retinopathy group versus proliferative diabetic retinopathy group, *p<0.05, †p<0.001.

diabetics without retinopathy (DC) as compared to the NC mean values. Yet, there is a near significant increase (p=0.06) in the mean concentration of glycated albumin in the PDR as compared to DC values in addition to a similar elevation in the glycated pre-β lipoprotein among diabetics with NPDR as compared to the NC values. A statistically significant increase was observed in the mean concentration of serum Cd and the ratio of Zn to Cu in DC as compared to NC mean values (p<0.05, p<0.001). The significant reduction (p<0.05) in serum Cr concentration displayed by all diabetic retinopaths is due to the highly significant depression in the mean serum Cr concentration (p<0.001) in those with NPDR as compared to the NC values. A highly significant decrease in the mean serum Cd concentration is observed only in PDR group as compared to both of the NC and DC values (p<0.001, p<0.05). The means of Zn/Cu ratios are significantly reduced (p<0.01) in all diabetic retinopathy groups as compared to DC mean values. Moreover, within the diabetic retinopathy groups, the serum mean Se level is found to be significantly lower in the PDR in comparison to those of PDR (p<0.001) whereas the mean serum Cd value is significantly higher in the NPDR group as compared to those of the sPDR group (p<0.001).

Table 3 reveals the effect of the duration of diabetes on the mean concentration of serum glycated albumin, glycated lipoproteins, and some trace elements in diabetic retinopaths and non-retinopaths. There is no significant changes in the mean concentrations of serum glycated albumin, alpha-lipoproteins, pre-ß-lipoprotein, and ß-lipoproteins among diabetic retinopaths with different duration of DM as compared to their respective values of diabetic controls. Yet, a statistically near significant decrease (p=0.06) in the mean serum glycated ß-lipoproteins was recorded in diabetic retinopaths who suffer from diabetes for up to 12 years (G2) as compared to values of those who have diabetes for <8 years (G1). Moreover, in the G1 group there is a significant increase in the mean serum Se (p<0.001) and a significant decrease in mean serum concentration of Cr, Cd, and Zn/Cu ratio (p<0.05, p<0.001) as compared to those of DC group. In G2 patients, there is a statistically significant decrease in the means of serum Cd (p<0.05), Se concentration, and Zn/Cu (p<0.001) in comparison to DC mean values. In patients with history of diabetes for more than 12 years (G3), there is a significant reduction in the mean serum Cr concentration (p<0.05) and more significant decrease in the mean of serum Zn/cu values (p<0.001) as compared to DC values. Furthermore, the mean concentration of Cr is significantly elevated (p<0.05) in G2 patients as compared with G3 values. Diabetic retinopaths within G3 group exhibit both significantly reduced mean serum Cr level, and highly increased mean serum Cd values (p<0.05, p<0.001) in comparison to those with shorter duration of diabetes (G1 group). Yet, statistically significant change (p=0.07) is observed in the mean serum Se concentration in the G2 versus G1 group.

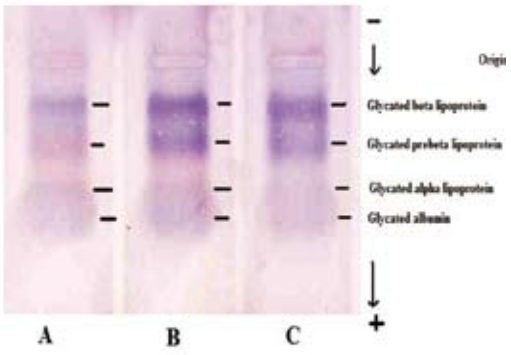

Figure 1 - Nitro blue tetrazolium-stained agarose gel electrophoreticogram of the glycated albumin and lipoproteins. Lane A: serum sample of normal control. Lane B: serum sample of patient with proliferative diabetic retinopathy. Lane C: serum sample of patient with nonproliferative diabetic retinopathy.

Table 3 - The effect of the different duration of diabetes on the mean concentration of the glycated albumin, glycated lipoproteins, and trace elements in diabetic retinopaths and non retinopaths.

Duration of diabetes	Glycated albumin (µM/l)	Glycated alpha-lipoprotein (µM/l)	Glycated Pre-ß lipoprotein (µM/l)	Glycated ß-lipoprotein (µM/l)	Selenium (µM/l)	Chromium (nM/l)	Cadmium (nM/l)	Zinc/Cu Ratio
Diabetic control (n=14)	30.57±4.816	21.49±2.358	22.73±3.791	27.15±4.306	23.65±21.106	100.8±36.058	0.58±0.897	1.31 ±0.181
G1 (n=8)	33.79±3.727	22.54±4.031	25.56±8.168	22.36±7.437	52.85[a†]±74.303	96.0[a*]±55.357	0.23[a*]±0.25	0.72 [a†]±0.26
G2 (n=11)	33.37±7.001	19.51±3.221	21.9±4.992	25.19±6.214	10.54[a†,b*]±9.819	101.2[b*]±18.02	0.20[a*]±0.259	0.82 [a†]±0.232
G3 (n=9)	32.83±5.119	19.126±6.624	21.8±3.082	25.84±6.942	27.79 ±20.83	65.07[a*,b*,c*]±42.059	0.54[c†]±0.802	0.72 [a†]±0.105

Results are expressed as mean ± standard deviation.
[a]student t-test: different groups (G1,G2,G3) versus diabetic controls: *p<0.05, †p<0.001. [b]student t-test: G2 versus G3: *p<0.05, †p<0.001.
[c]student t-test: G1 versus G3: *p<0.05, †p<0.001.
G1 - (0.1-8 years) encompass those who have diabetes mellitus for up to 8 years. G2 - (8.1-12 years) include patients who suffer from diabetes for more than 8 years but less than 12 years. G3 - (>12 years) involves diabetic retinopaths with a history of diabetes mellitus that exceeds 12 years duration.

Figure 2 reveals the association between the concentrations of serum glycated albumin, glycated lipoproteins, some trace elements, and the duration of diabetes in diabetic retinopaths. The serum concentrations of the glycated albumin and glycated alpha-lipoproteins are inversely correlated (**Figures 2a & 2b**) with both of the serum glycated pre-ß lipoprotein and glycated ß-lipoprotein values. The serum glycated ß-lipoprotein shows a significant positive relationship (**Figure 2b**) with serum glycated pre ß-lipoproteins and a significant inverse relationship (**Figure 2c**) with the serum Se values. The serum Cd level correlated positively with the fasting serum glucose values in patients with diabetic retinopathy and the Cd level rises with the increase in

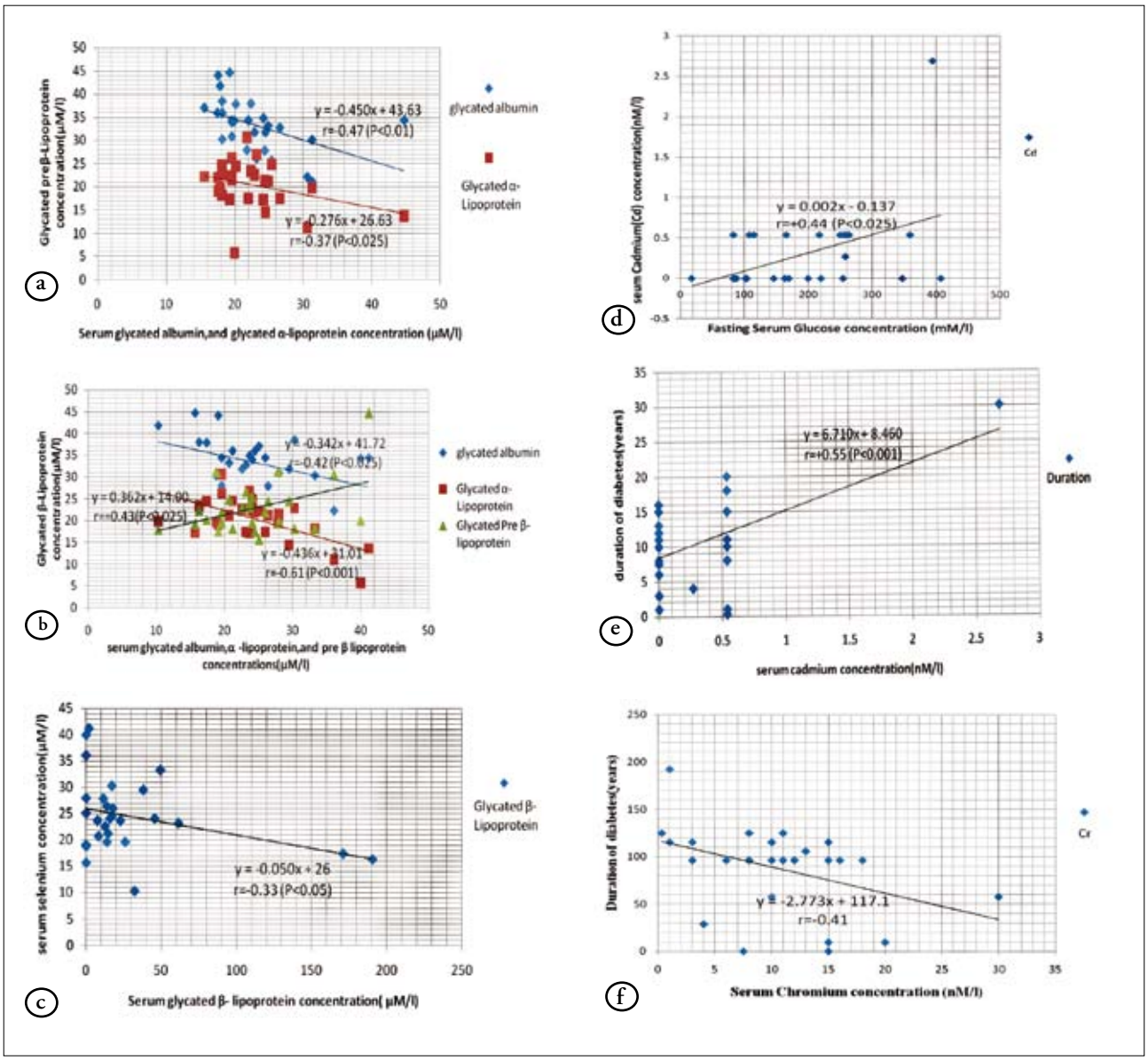

Figure 2 - Trace elements interrelation with albumin and lipoprotein glycation. **a**) The correlation of the serum glycated pre ß-lipoprotein concentration with the serum glycated albumin (p<0.01) and glycated alpha-lipoprotein concentrations (p<0.025) in patients with diabetic retinopathy. **b**) The correlation of the serum glycated ß-lipoprotein concentration with the serum glycated albumin (p<0.025), glycated ß-lipoprotein (p=0.001) and pre alpha-lipoprotein concentrations (p<0.025) in patients with diabetic retinopathy. **c**) The correlation of the serum Selenium concentration (p<0.05) with the serum glycated ß-lipoprotein concentrations in patients with diabetic retinopathy. **d**) The correlation of the serum cadmium concentration (p<0.025) with the fasting serum glucose concentration in patients with diabetic retinopathy. **e**) The correlation of the serum cadmium concentration (p<0.001) with the duration of diabetes mellitus in patients with diabetic retinopathy. **f**) The correlation of the serum chromium concentration (p<0.025) with the duration of diabetes mellitus in patients with diabetic retinopathy.

the duration of diabetes (**Figures 2d & 2e**). On the other hand, the serum Cr values in these patients (**Figure 2f**) decrease with the prolongation of diabetes.

Discussion. Diabetic retinopathy poses a serious threat to vision. Overall, diabetic retinopathy is estimated to be the most frequent cause of new cases of blindness among adults aged 20-74 years.[11] Increased oxidative and glyco-oxidative stress has been implicated in the pathogenesis of diabetic complications, including nephropathy.[12] In this study, significant elevation is observed only in the glycated ß-lipoprotein among diabetics without retinopathy. Yet, the near significant increase in the glycated pre ß-lipoproteins in the NPDR and similar difference in the glycated albumin in diabetics with the PDR is probably due to the small number of the studied patients within each group. This finding is in agreement with the observations of Yegin and Ozben[13] who reported increased levels of all glycated lipoproteins in diabetic patients with and without vascular complications, with no significant differences between diabetic patients with and without complications except for glycated very low density lipoprotein (VLDL) (glycated pre ß-lipoproteins). However, Kobayashi et al[10] demonstrated raised glycated LDL (glycated ß-lipoproteins) values in diabetic patients with complications compared with those without. Diabetic patients with complications had higher concentrations of glycated ß-lipoproteins, with large individual variations, than patients without complications. The greatest concentration (1.02 mmol/L) being found in patients with diabetic retinopathy and/or nephropathy. The concentration of glycated ß-lipoprotein (glycated LDL) in serum seems to depend on the extent and duration of hyperglycemia; it may also be a useful diagnostic indicator of diabetic atherogenesis, microangiopathy (namely retinopathy or nephropathy), and other complications.[10] Furthermore, the susceptibility of LDL to oxidation was strongly correlated with degree of LDL glycosylation[14] and the ratio of vitamin E/lipid peroxide of LDL.[15] Panteghini et al[16] reported that the mean concentration of glycated Apo B in 45 non-diabetic subjects was 4.3% ± 1%. In 17 patients with type 1 and in 60 patients with type 2 diabetes the mean glycated Apo B concentrations were 5.3% ± 0.7% and 5.9% ± 1.1%, and were significantly higher than in the controls ($p<0.001$). It was hypothesized that retinal capillary leakage during the early stage of diabetic retinopathy enables LDL to be extravasated and trapped in the extra vascular and sub-endothelial spaces, and that subsequent glycation and oxidation of extravasated LDL under hyperglycemia and enhanced oxidative stress lead to retinal vascular injury.[17,18] These notions are supported by previous studies, which showed HOG-LDL significantly induced apoptosis in cultured bovine retinal capillary endothelial cells and pericytes, and in human retinal capillary pericytes (HRCP), and induced many alterations in gene expression and function in HRCP.[3] The underlying mechanisms by which HOG-LDL may trigger pericyte loss include induction activation of caspase pathways, and mitochondrial dysfunction.[19] Approximately 10% of the albumin in normal human serum is modified by nonenzymatic glycosylation primarily at the epsilon-amino group of lysine residue 525. The non-enzymatic glycosylation induces a conformational change in human serum albumin.[11] Eaton and Qian[20] reported that glycated albumin binds approximately 3-fold greater amounts of both Cu and iron and the Cu bound to glycated albumin remains redox active (namely capable of supporting the oxidation of ascorbic acid). These findings support the hypothesis that glycated proteins bind transition metals such as Cu and iron, and that such 'glycochelates' accumulate within the vasculature in diabetes and catalytically inactivate endothelium derived relaxing factor (EDRF).[20]

Our finding of the significant inverse correlation between the glycated albumin and glycated pre ß-lipoprotein and ß-lipoprotein reinforces the fact that albumin acts as a radical trapping and metal sequestering agent, and the glycated albumin is easily prone to metal oxidation by this it protects and delays the LDL and VLDL glycation and oxidation. Bovine serum albumin (BSA) might work either as a radical sink, thus protecting the protein from entire denaturation, or as agents transferring the damage to other sites such as the peptide backbone. This may explain why in some instances, BSA not only lost its antioxidant effect but also became pro-oxidant.[21] In this study, we observed that the rise in the concentration of glycated ß-lipoprotein is associated with the reduction in the concentration of the glycated pre ß- and ß-lipoprotein in diabetics with retinopathy. This finding probably reflects the protective role of the glycated ß-lipoprotein (glycated high density lipoprotein, HDL) against the glycation of the ß-lipoprotein (glycated LDL) and pre ß-lipoprotein (glycated VLDL) fractions. Although Solakivi et al[22] reported that an in vitro HDL does not protect LDL from oxidation, but in fact it oxidized fastest of all lipoproteins due to its fatty acid composition, which is oxidation promoting. The LDL from diabetic subjects is more susceptible to oxidation than LDL from non-diabetic subjects. Supplementation of diabetic subjects with antioxidant vitamins significantly decreases susceptibility of LDL to oxidation by Cu.[23] The maximal peroxidation rate (Vmax) of HDL and

LDL depended similarly on the molar ratio of bound Cu/lipoprotein. Such results imply that the maximal surface density of bound Cu is at least 2-fold higher for HDL than for LDL. This difference may be responsible for the higher susceptibility of HDL to Cu-induced oxidation in the presence of high Cu concentrations.[24] Moreover, the reduction of the binding of Cu to LDL by competitive binding to the HDL also contributes to the antioxidative effect of HDL.[25] Nonetheless, our result is more in line with those obtained in well-controlled type 1 DM, where glycated LDL provides a longer lag time than that of non-glycated LDL.[26] On the contrary, Jenkins et al[26] reported that in 548 men with type 1 DM retinopathy was positively associated with small LDL, LDL particle concentration, apo-ß concentration and small HDL and negatively associated with large LDL, LDL size, large HDL, and HDL size. No associations were found with apoA1, Lp(a), or susceptibility of LDL to oxidation.[27] The decrease in the mean Zn/Cu ratios recorded in our study in all diabetic retinopaths reflects the increase in the serum Cu and a concomitant increase in the mean serum Zn concentration (data are not shown) in comparison to diabetics without retinopathy. A similar finding was reported by Craft et al[28] in diabetic rats with retinopathy, hypertension, and macrovascular disease. Several complications of diabetes may be related to increased intracellular oxidants and free radicals associated with decreases in intracellular Zn and in Zn dependent antioxidant enzymes.[29] Zinc and Cu are necessary factors in a variety of "antioxidant" enzymes, particularly superoxide dismutase, catalase, and peroxidase. In an animal model, Nicolas et al[30] detected a reduction of 60% in catalase and glutathione peroxidase activities in the affected retinas. The affected retinas also showed 4-fold lower Zn concentration compared with the normal controls. This correlates with the lowered metallothionein expression. And since metallothionein is suggested to function as a free radical scavenger, the lowered metallothionein synthesis may consequently contribute to increased peroxidation reactions in the affected retinas.[30] Faure et al[31] reported that Zn supplementation caused a decrease in lipid peroxidation and an increase in Se-GPx activity in insulin-dependent diabetic patients with retinopathy, which could be linked to the protective effect of Zn on the protein itself. We found that the serum mean values of Zn is higher in normal and diabetics without retinopathy than diabetics who have retinopathy (as reflected by an increase in the Zn/Cu ratio in these patients). This finding is inline with those of Vulpe et al,[32] who reported an increase in Zn level in diabetic patients with no retinopathy (p=0.05). On the contrary, Williams et al[33] recorded that plasma Zn was 17% lower in the diabetic group than the control group (p<0.001), but the intracellular Zn differences did not reach statistical significance suggesting that the renal effects might be predominant in the eventual production of the total body Zn depletion in diabetes. We reported a decrease in the mean serum Se concentration in PDR group as compared to those of the NPDR group. This reduction is in concomitant with the persistence hyperglycemia and uncontrolled diabetes in these patients (serum fructosamine concentration: 474.7 Micromole/l). This observation reinforces the role of Se in regulating glucose concentration in vivo. This insulin like action of Se includes stimulation of glucose uptake and regulation of metabolic processes such as glycolysis, gluconeogenesis, fatty acid synthesis, and pentose phosphate pathway.[34] The decrease in the serum Se values is found to be significantly associated with the increase in the level of the serum glycated ß-lipoprotein concentration as Se is an important co-factor for the Se dependent peroxidase, which detoxify a wide variety of peroxides including lipid derived-species (LOOHs) present in oxidized LDL, thus Se treatment can ameliorate the modification occurring in LDL-cholesterol by oxidative injury.[35] Furthermore, susceptibility of LDL to oxidation was strongly correlated with degree of LDL glycosylation.[14] Like other serum proteins, lipoproteins are non-enzymatically glycated in the presence of glucose.

Apo lipoprotein B100 of LDL, usually has some of its lysine residues modified by non-enzymatic glycation. Glycation of Apo-B100 also slows the catabolism of LDL.[4] Glycated LDL caused a significant increase in platelet nitric oxide production, intracellular Ca2 concentration, and aggregating response to adenosine diphosphate (ADP); an inhibition of the platelet membrane sodium, potassium adenosinetriphosphatase (Na-K-ATPase) activity; and a stimulation of calcium-dependent adenosine triphosphatase (Ca2-ATPase) activity.[36] An intra retinal immunofluorescence of the Apo B100 and oxidized LDL in the retinal sections of non-diabetics, type 2 DM without retinopathy, diabetics with moderate NPDR and diabetics with PDR revealed that the intra retinal oxidized glycated LDL increases with the severity of diabetic retinopathy.[3] The mean serum Cr was found to be significantly reduced in all diabetic retinopaths with more augmented depression in the NPDR group. This reflects a second factor for the persistent hyperglycemia in these patients as Cr is involved in the insulin signaling cascade that leads to increase in the glucose utilization by the peripheral tissues.[37] Moreover, the depression recorded in the Cr concentration in diabetics with retinopathy is found to increase progressively with the duration of type 2 DM.

In this study, a significant increase in the mean serum Cd, Zn/Cu ratio, and a non significant reduction in the mean serum Se concentration was observed among diabetics without retinopathy. This raised Zn/Cu ratio may have a protective effect for the retinal pericytes against Cd toxicity since Cd was reported to induce changes in the intracellular distribution of Cu, Zn, Mn, and Se.[38] Whereas in diabetics who develop NPDR, the protective role of Zn on the high Cd concentration is lost due to the low Zn/Cu ratio in the presence of an elevated level of serum Cd. Yet, the normal serum Se values in these patients may confer some protection to their retinas against progression to proliferative retinopathy. Jamall and Roque,[39] reported that feeding rats 50 ppm Cd for just 7 weeks resulted in detectable levels of Cd in their eyes. Furthermore, these ocular Cd concentrations cause significant alterations in the levels of the essential trace elements Se, calcium, iron, and Cu in rat eyes. Although different mechanisms lead to the production of reactive oxygen species by Cr and Cd, similar subsequent mechanisms and types of oxidative tissue damage are involved in the overall toxicities. These include increased lipid peroxidation, enhanced excretion of urinary lipid metabolites, modulation of intracellular oxidized states, DNA damage, membrane damage, altered gene expression, and apoptosis, enhanced production of nuclear factor-kappa-beta, and activation of protein kinase C and p53 tumor suppressor gene.[40]

Increasing rates of type 2 DM worldwide suggest that diabetes may be caused by environmental toxins.[6] Cadmium is known to accumulate in the human pancreas.[41] Pancreatic dysfunction was reported in smelter workers who had been exposed to Cd for more than one year. The endocrine function of the pancreas was affected at lower urinary levels of Cd, whereas the exocrine function was seen at higher urinary levels of Cd than those providing increase to renal tubular dysfunction.[42] In agreement to this research, we found that the serum Cd level exhibits direct significant association with the serum fasting glucose concentration. Furthermore, the positive correlation between the duration of diabetes and the serum Cd levels in diabetics with retinopathy and the absence of this association in those without retinopathy sheds light on the role of Cd in the pathogenesis, development, and the progression of retinopathy in patients with type 2 DM. This observation sets the direction for further research and investigations on larger samples using modern tools.

The concentration of Cr and all of the studied minerals in the 24-hour urine volume was not carried out, although this is very important as in DM the glucose-mediated hyper osmotic glomerular filtrate may be largely responsible for the enhanced urinary mineral loss and adjustment of all values to gram creatinine would provide more reasonable clues. The number of patients and controls was inadequate to achieve significant results as multiple comparisons were carried out because some patients drift through the area and others were seen intermittently. Likewise, glycosylated hemoglobin (HbA1c) test was not available for all patients to check the effect of glycemic control on the studied parameters. Further studies are recommended to test the impact of these minerals on the oxidizability of both HDL and LDL sub-fractions in different forms of diabetic retinopathy.

In conclusion, the persistent hyperglycemia is in concomitant with the hypo mineral status observed in diabetic retinopaths and the metal catalyzed oxidation of the glycated lipoproteins could shed light on the causal relationship between the fore mentioned minerals and the development and progression of diabetic retinopathy.

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