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Study of the level of Copeptin in patients with Diabetic Retinopathy

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ABSTRACT

Background: The prevalence of Diabetic retinopathy in Egypt among adult diabetic patients in 2010 was around 20.5%. 90 percent of all cases of blindness from diabetes can be prevented. Copeptin, a novel biomarker (a surrogate to arginine vasopressin was found to increase with diabetic nephropathy.

Objectives: to study the level of copeptin in patients with diabetic retinopathy.

Methods: The study was conducted on 96 individuals divided into 4 groups. **Group I** 24 patients with proliferative diabetic retinopaty (PDR), **Group II** 24 patients with non proliferative diabetic retinopathy (NPDR), **Group III** 24 diabetic patients with no evidence of retinopathy and **Group IV** 24 healthy non diabetic individuals. All participants were subjected to full medical history taking, slit lamp biomicroscope fundus examination and measurement of serum fasting blood sugar, 2 hour post prandial blood sugar, glycated hemoglobin, serum creatinine, serum copeptin and urinary albumin creatinine ratio.

Results: the study shows a statistically significant rise in the level of copeptin in patients with PDR **(Group I)** and NPDR **(Group II)** when compared with those with no diabetic retinopathy **(Group III)** and the control group **(Group IV)** P value < 0.001. There was a statistically significant positive correlation with duration of diabetes (r = 0.589) and level of albumin/creatinine ratio (ACR) (r = 0.540) P value < 0.001.

Conclusion: : Copeptin was found to be higher in proliferative diabetic retinopathy and non proliferative diabetic retinopathy when compared to diabetics with no retinal complications and healthy individuals with a statistically significant difference. It was found to be significantly higher in diabetic patients in comparison with the normal population. There was a statistically significant positive correlation with ACR and duration of diabetes.

Keywords: Copeptin, proliferative diabetic Retinopathy, non proliferative diabetic retinopathy, diabetes mellitus, biomarkers

INTRODUCTION

Diabetes Mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with damage, dysfunction and failure of different organs [(1).] Diabetic retinopathy is the leading cause of blindness in patients aged 20 to 74 years. Chronic hyperglycemia affects the retinal vessels resulting in diabetic retinopathy. The risk of development and progression of diabetic retinopathy is closely associated with the type and duration of diabetes [(2).] Copeptin is the C-terminal part of the vasopressin prohormone**.** It is relatively stable in the serum and its levels can be measured accurately. It is equimolar to vasopressin in secretion and hence directly reflects its serum levels. It could be used as an early and adequate marker for organ damage[(3)]A study done in Denmark revealed a positive correlation between serum copeptin levels and renal impairment and peripheral arterial disease in type 2 diabetic patients[(4)]**.** This study aimed to detect levels of copeptin in patients with diabetic retinopathy.

PATIENTS AND METHODS

Any patient aged 20-60 years with diabetes and no other co-morbidities was included in the study.

This study included 96 individuals and they were divided into 4 groups. **Group I** consisted of 24 patients with proliferative diabetic retinopathy

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(PDR), **group II** 24 patients with non proliferative diabetic retinopathy (NPDR), **group III** included 24 diabetic patients with no fundus abnormalities and **group IV** included 24 healthy non diabetic individuals. The individuals were subjected to full medical history taking, slit lamp biomicroscope fundus examination and the measurement of serum fasting blood sugar, 2 hour post prandial blood sugar, glycated hemoglobin, serum creatinine, serum copeptin and urinary albumin creatinine ratio. History taking included age, gender, type of diabetes, duration of diabetes, antidiabetic medications, any other medical comorbidities and pregnancy for females.

Patients were excluded from this study if they had: Evidence of diabetic nephropathy, chronic liver disease, ischaemic heart disease, cerebrovascular stroke or hypertension. Extremes of age and pregnant ladies were all excluded.

The study was approved by the Ethics Board of Ain-Shams University.

Statistical analysis

The SPSS 10.0 for windows was used for data management and analysis and the Microsoft power point for charts. Quantitative data were presented as mean +SD. For the comparison of the groups' means, one way analysis of variance (ANOVA) was used and the F value was calculated. Non parametric quantitative data were expressed as median (range). Tukey's tests were used for comparison of means. Qualitative data was expressed as frequency and percentage. Association between qualitative data was done using Chi-square test. P value was considered highly significant at <0.001. The correlation coefficient r was used to measure the strength and direction of a linear relationship between two variables on a scatter plot.

RESULTS

Comparing the demographic data of all groups: (table1): there was no statistical difference among all 4 groups regarding gender. As regards the gender of the studied population we found 32 subjects (33.33%) to be males, while 64 subjects (66.67%) were females. In-group I and III 25 % were males while 75 % were females. While in group II 33.3% were males while 66.7 % were females. In group IV 12 (50%) were males and 12 (50%) were females**.**

Comparing the clinical data of all groups: (table1) :There was a significant difference among the groups regarding the prevalence of diabetic retinopathy in relation to the type of diabetes. It was higher in type 2 diabetes patients. In our study 18 subjects (25%) were type 1, while 54 subjects (75%) were type 2. In group I 4 individuals (16.67%) were type I whereas 20 subjects (83.3%) were type 2. In group II none of the subjects were type I and hence all 24 subjects (100%) were type 2. As for group III 14 subjects (58.3%) were type 1, while 24 (41.6%) were type 2. Regarding the duration of diabetes there was no significant difference among the 4 groups, being15.58±4.916 years for group I, 11.37±3.29 years for group II and 13.3±5.73 years for group III. Group IV were healthy non diabetic individuals. Furthermore, there was a significant difference among all 4 groups regarding FBS, 2hrPP and HbA1c. For FBS and HbA1c, the highest means are for group I, lower in group II, even lower in group III and lowest in group IV. Regarding the FBS, the means were 232.167±43.139mg/dL for group I, 210.292±46.617mg/dL for group II, 147.667±79.469mg/dL for group III and 86.375±12.744mg/dL for group IV. As regards the 2hr PP, the means were 304.542±52.778mg/dL for group I, 310.583±71.671mg/dL for group II, 222.000±78.501mg/dL for group III, and 102.254±24.649mg/dL for group IV.In our study, the values of HbA1c were as follows: for group I the mean was 9.863±0.976%. As for group II mean was 9.829±1.259%. For group III the mean was 9.0±2.498%. As for the controls, the mean was 5.08±0.299%. There was a non significant difference between all groups regarding the serum creatinine. While, the mean for group I was 0.913±0.233mg/dL, for group II, the mean was 0.933 ±0.218 mg/dL. As for group III the mean was 0.883± 0.143mg/dL. For the controls (group IV), the mean was 0.829±0.212 mg/dL. The ACR was significantly different among the different groups. It was highest in group I (mean of 139.583±31.554), lower in group II (mean of 133.167±48.080), and even lower in group III (120.333±40.051) and lowest in group IV (mean of 24.583±3.035). Regarding the level of serum copeptin, there was a significant difference between the groups, being highest for group I (39.633±3.919pmol/L), lower for group II (34.946 ±4.869pmol/L), even lower for group III (27.779±10.529pmol/L) and lowest for group IV (7.421±4.243pmol/L). Regarding the level of

copeptin among the different groups, there was a non significant difference in relation to gender, type of diabetes, FBS, 2hr PP, HbA1c and serum creatinine. However serum copeptin was found to be significantly different among the different groups in relation to ACR and duration of diabetes. Being higher as these parameters increased in value. **(Figures 1 & 2).** There is no significant difference between Group I and Group II regarding any parameter. **(Table 2).** There a significant difference between Group I and Group III regarding FBS, 2hr PP and serum copeptin, being higher in group III for all parameters. **(Table 3).**There is a significant difference between Group I and Group IV regarding all parameters being higher in Group I. Serum creatinine is the only exception. No significant difference is noted between both groups for this parameter. **(Table 4)** There a significant difference between Group II and Group III regarding FBS and 2hr PP being higher in group II for all parameters. **(Table 5)** There is a significant difference between Group II and Group IV regarding all parameters being higher in Group II. Serum creatinine is the only exception. No significant difference is noted between both groups for this parameter. **(Table 6).** There a significant difference between Group III and Group IV regarding FBS, 2hr PP and serum copeptin, being higher in group III for all parameters. **(Table7)** Regarding the medications of the diabetic individual in the study, 12 were on oral hypoglycemic drugs only, 38 were on insulin only and 22 were on both oral hypoglycemic drugs and insulin.

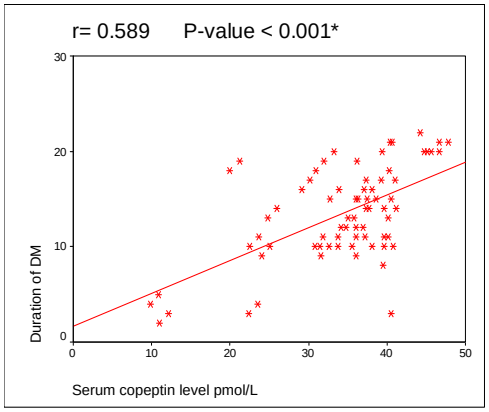

Figure 1: showing a line graph between serum copeptin and duration of diabetes

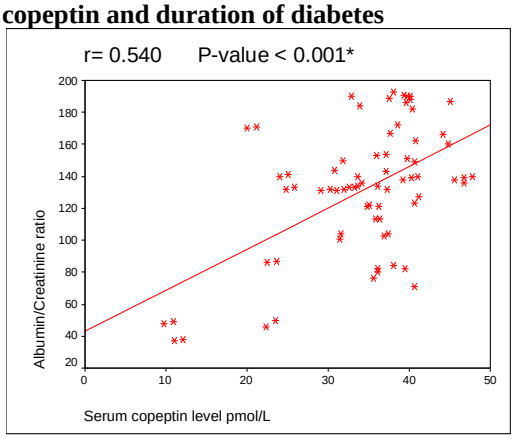

Figure 2: showing a line graph between serum copeptin and ACR.

Table 1: comparing demographic and clinical data of all groups

Variant	PDR (group I)	NPDR (group II)	No DR (group III)	Controls (group IV)	P-value
Gender:Male	25%	33.3%	25%	50%	0.212
Female	75%	66.7%	75%	50%	
Type of DM:Type I	16.67%	0%	58.33%		<0.001
Type 2	83.33%	100%	41.67%		
Duration of Diabetes	15.583±4.916	11.375±3.294	413.250±5.728		0.012
FBS	232.167±43.139	210.292±46.617	147.667±79.469	86.375±12.744	<0.001
2hrPP	304.542±52.778	310.583±71.671	222.000±78.501	102.254±24.649	<0.001
HbA1c	9.863±0.976	9.829±1.259	9.000±2.498	5.083±0.299	<0.001
creatinine	0.913±0.233	0.933±0.218	0.883±0.143	0.829±0.212	0.324
ACR	139.583±31.554	133.167±40.080	120.333±40.051	24.583±3.035	<0.001
Copeptin	39.633±3.919	34.946±4.869	27.779±10.529	7.421±4.243	<0.001

PDR (Group I) = proliferative diabetic retinopathy
NPDR (Group II) = non proliferative diabetic retinopathy
No DR (GroupIII) = no diabetic retinopathy
Controls (Group IV) = healthy non diabetic individuals
HbA1c = glycated hemoglobin
2hr PP = 2 hour post prandial blood sugar
ACR = albumin creatinine ratio
FBS = fasting blood sugar

There was no significant difference among the groups regarding gender, duration of diabetes and serum creatinine. However there was a significant difference among the groups regarding the remaining parameters.

Table 2: comparing demographic and clinical data of group I and group II.

Parameter	Group I (PDR)	Group II (NPDR)	P value
Duration of diabetes	15.583±4.916	11.375±3.294	0.009
FBS	232.167±43.139	210.292±46.617	0.455
2hr PP	304.542±52.778	310.583±71.671	0.986
HbA1c	9.863±0.976	9.829±1.259	1.000
Serum creatinine	0.913±0.233	0.933±0.218	0.074
ACR	139.583±31.554	133.167±48.080	0.921
Copeptin	39.633±3.919	34.946±4.869	0.066

There is no significant difference between Group I and Group II regarding any parameter.

Table 3: comparing demographic and clinical data of group I and group III.

Parameter	Group I (PDR)	Group III (No DR)	P value
Duration of diabetes	15.583±4.916	13.250±3.294	0.213
FBS	232.167±43.139	147.667±79.469	<0.001
2hr PP	304.542±52.778	222.000±78.501	<0.001
HbA1c	9.863±0.976	9.000±2.498	0.193
Serum creatinine	0.913±0.233	0.883±0.143	0.111
ACR	139.583±31.554	120.333±40.051	0.235
Copeptin	39.633±3.919	27.779±10.529	<0.001

There a significant difference between Group I and Group III regarding FBS, 2hr PP and serum copeptin, being higher in group III for all parameters.

Table 4: comparing demographic and clinical data of group I and group IV.

Parameter	Group I (PDR)	Group IV(Controls)	P value
FBS	232.167±43.139	86.375±12.744	<0.001
2hr PP	304.542±52.778	102.254±24.649	<0.001
HbA1c	9.863±0.976	5.083±0.299	<0.001
Serum creatinine	0.913±0.233	0.829±0.212	0.310
ACR	139.583±31.554	24.583±3.035	<0.001
Copeptin	39.633±3.919	7.421±4.243	<0.001

There is a significant difference between Group I and Group IV regarding all parameters being higher in Group I. Serum creatinine is the only exception. No significant difference is noted between both groups for this parameter.

Table 5: comparing demographic and clinical data of group II and group III.

Parameter	Group II (NPDR)	Group III (No DR)	P value
Duration of diabetes	11.375±3.294	13.250±3.294	0.364
FBS	210.292±46.617	147.667±79.469	<0.001
2hr PP	310.583±71.671	222.000±78.501	<0.001
HbA1c	9.829±1.259	9.000±2.498	0.223
Serum creatinine	0.933±0.218	0.883±0.143	0.324
ACR	133.167±48.080	120.333±40.051	0.586
Copeptin	34.946±4.869	27.779±10.529	0.001

There a significant difference between Group II and Group III regarding FBS and 2hr PP being higher in group II for all parameters.

Table 6: comparing demographic and clinical data of group II and group IV.

Parameter	Group II (NPDR)	Group IV (Controls)	P value
FBS	210.292±46.617	86.375±12.744	<0.001
2hrPP	310.583±71.671	102.254±24.649	<0.001
HbA1c	9.829±1.259	5.083±0.299	<0.001
Serum creatinine	0.933±0.218	0.829±0.212	0.383
ACR	133.167±48.080	24.583±3.035	<0.001
Copeptin	34.946±4.869	7.421±4.243	<0.001

There is a significant difference between Group II and Group IV regarding all parameters being higher in Group II. Serum creatinine is the only exception. No significant difference is noted between both groups for this parameter.

Table 7: comparing demographic and clinical data of group III and group IV.

Parameter	Group III (No DR)	Group IV (Controls)	P –value
FBS	147.667±79.469	86.375±12.744	<0.001
2hrPP	222.000±78.501	102.254±24.649	<0.001
HbA1c	9.000±2.498	5.083±0.299	0.193
Serum creatinine	0.883±0.143	0.829±0.212	0.111
ACR	120.333±40.051	24.583±3.035	0.235
Copeptin	27.779±10.529	7.421±4.243	001

There a significant difference between Group III and Group IV regarding FBS, 2hr PP and serum copeptin, being higher in group III for all parameters.

DISCUSSION

Diabetic retinopathy is the leading cause of vision loss in adults aged 20-74 years [5].

Diabetic retinopathy ranked as the fifth most common cause of preventable blindness and the fifth most common cause of moderate to severe visual impairment from 1990 to 2010[6]. In 2010, 285 million people worldwide were estimated with diabetes, over one-third had signs of diabetic retinopathy and a third of these were afflicted with vision-threatening diabetic retinopathy, defined as severe non-proliferative diabetic retinopathy or proliferative diabetic retinopathy or the presence of diabetic macular edema [7].

Proliferative diabetic retinopathy is the most common vision-threatening lesion particularly among patients with type 1diabetes.[8].

One of the prime motivating factors behind the development of a screening program for diabetic retinopathy is the efficacy of laser photocoagulation treatment in preventing vision loss. The beneficial effect of laser treatment was established by two large randomized clinical trials—the Diabetic Retinopathy Study and the Early Treatment Diabetic Retinopathy Study (ETDRS). The essential findings of these trials were that, compared with no treatment, laser photocoagulation prevented vision loss in patients with proliferative diabetic retinopathy and macular oedema by about 50%. [9]

Biomarkers are being studied in relation to diabetic retinopathy and they include angiopoetin 1. A study done in 2015 revelaed that there is a significant reduction in serum Angiopoetin 1 in patients with DR and is correlated with the severity of DR. This reduction of Ang1 may reflect a role in the pathogenesis and progression of DR. [10].

Copeptin, a 39-amino acid glycopeptide that comprises the C-terminal part of the AVP precursor (CT-proAVP), was found to be a stable and sensitive surrogate marker for AVP release. Copeptin measurement has been shown to be useful in various clinical indications. Arginine vasopressin (AVP) is a key hormone in the human body. Despite the clinical relevance of AVP in maintaining fluid balance and vascular tone, measurement of mature AVP is difficult and subject to preanalytical errors hence copeptin is a reliable substitute [11]. In recent years copeptin is being studied as a diagnostic and prognostic biomarker in various

diseases[12]. The aim of our study was to find the level of serum copeptin in patients with diabetic retinopathy.There was a statistically significant increase in copeptin level in patients with diabetes mellitus and further increase in patients with diabetic retinopathy. Copeptin was found to be significantly higher in Group II (NPDR) in comparison to Group III (no DR). Enhorning S mentioned and explained that copeptin could be used as a predictor for the occurrence of diabetes mellitus. This is because AVP mediates liver glycogenolysis through V1a receptor. This may explain why it is higher in diabetics in comparison to healthy individuals even in the absence of diabetic complications.[13] It was observed that copeptin levels were highest in group I (PDR) and group II (NPDR) with a statistically significant difference when compared to Group III (no DR) and Group IV (control group). Recently, **Boertien et al** demonstrated that copeptin predicts the estimated glomerular filtration rate decline in subjects with type 2 diabetes. The retina being a vascular bed also affected by diabetes may experience similar microvascular changes as the kidney. This may explain why copeptin is highest in group I with a statistically significant difference, which is the study group with the most severe form of diabetic retinopathy.[14] Another study done by Wiebke et al revealed that copeptin levels predicted the development of ESRD in diabetic patients. Copeptin is correlated with ultrafiltration volume, suggesting that volume depletion during dialysis might be responsible for an increase in AVP concentrations also. Furthermore, resistance of V2 receptors is present already at earlier stages of chronic kidney disease, probably involving activation of feedback mechanisms with a regulatory increase of plasma AVP levels. These findings suggest that high copeptin levels may also reflect AVP action in ESRD and hence in diabetic retinopathy.[15] In December 2016, copeptin levels were found to be higher in patients with diabetic retinopathy in the Chinese population which further supports our findings although the exact mechanism remains unclear.[16] There was a statistically significant positive linear correlation between the value of ACR and the severity of the diabetic retinopathy, being least in the control group (group IV) and highest in the group complaining of PDR (group I). Similarly, in a study by Padmaja KR, albuminuria matches the presence of diabetic retinopathy.[17] In another study by Meijer et al, plasma copeptin was shown to be positively associated with the prevalence of microalbuminuria in cross-sectional observational and long-term follow-up studies in the general population. The reason behind this was thought to be that vasopressin stimulates V2 receptor leading to increased urinary albumin excretion.[18]We also found that copeptin level was independent of the type of diabetes. And so far we did not find any study that compares the type of diabetes to copeptin level. We found that copeptin level was not related to patient gender which is inconsistent with a study conducted by Sanjay SB which reported higher level in males.[19] There was a statistically significant positive correlation between duration of diabetes and serum copeptin level. This is supported by **Fadia T** who reported that duration of diabetes is related to development of diabetic retinopathy. Duration of diabetes was identified as a risk factor for the development of diabetic complications.[20]Furthermore, the levels of FBS and HbA1c were highest in the group with PDR, lower in the group with NPDR and lowest in the group with no DR with a significant statistical difference. This agrees with the study by **Fadia T**, which states that poor glycemic control is related to the development and the degree of diabetic retinopathy. This is because persistent hyperglycemia was found to be a risk factor for the occurrence of diabetic complications.**[20]** In our study the range of ages varied from 20-60 years old. In those with diabetic retinopathy, the ages ranged from 43-60 years. This is supported by the study by **Congdon NG**, which mentioned that diabetic retinopathy is the most common cause of vision loss among people with diabetes and a leading cause of blindness among working-age adults and those aged 20-64.[21]

Study Limitations: The number of individuals was not conclusive being only 96 individuals. There was probably a selection bias due to the place of sampling being a morning outpatient

clinic of a governmental hospital. Collecting the samples was very difficult due to the exclusion criteria. A very few people develop diabetic retinopathy without developing other complications and without having other comorbidities.

CONCLUSION: Copeptin was found to be higher in proliferative diabetic retinopathy and non proliferative diabetic retinopathy when compared to diabetics with no retinal complications and healthy individuals with a statistically significant difference. It was found to be significantly higher in diabetic patients in comparison with the normal population. There was a statistically significant positive correlation with ACR and duration of diabetes.

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