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Evaluation of Plasma Endothelin-1 and Serum Inflammatory Markers in Patients with Diabetic Retinopathy

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Hyperglycemia has been identified as the major factor for the development of diabetic microvascular complication including retinopathy. Increased expression of Endothelin -1 (ET-1) is associated with diabetic retinopathy and vasculopathy, although the molecular explanation has not been defined. The study was conducted on 55 patients suffering from type 2 diabetes attending the Retina Clinic of Ophthalmology, Research Institute of Ophthalmology, seeking advice as regard eye complications of diabetes. The patients were divided into three groups: Group I included 15 patients who had no retinopathy, Group II including 20 patients with non-proliferative diabetic retinopathy of varying severity and Group III including 20 patients with proliferative diabetic retinopathy. Beside 20 healthy, age and sex matched subjects as a control group. The aim of this study was to examine whether ET-1 plays a role in the progression of diabetic retinopathy in cooperation with the other inflammatory markers. Also to detect the relationship between the stages of diabetic retinopathy and the inflammatory activity by measuring serum IL-6, CRP, haptoglobin and alpha-1 antitrypsin as markers of inflammation. All patients were subjected to the following: detection of plasma levels of glucose and ET-1, serum levels of IL-6, CRP, haptoglobin, α-1 antitrypsin, ALT and AST). The results showed that, the plasma level of ET-1 was increased significantly in-Group II and Group III as compared to the control group and the highest level was detected in-Group III. While, the other inflammatory markers (IL-6, CRP, haptoglobin and α-1 antitrypsin) its serum levels were increased significantly as compared to the control group. There were statistically significant positive correlation ($p<0.05$) between ET-1 and both of the IL-6 and CRP ($r = 0.389$ and 0.623, respectively). The best cut off values of ET-1 in Group I was 1.32 pg mL^{-1} with sensitivity 80% and specificity of 90%, while in Group II the best cut off value was 1.51 pg mL^{-1} with sensitivity 95% and specificity of 100% and in Group III the best cut off value was 1.63 pg mL^{-1} with sensitivity 100% and specificity of 100%. The positive and the negative predictive values in the three studied groups were (90.9 and 82.6, 100 and 95.24 and 100 and 100%, respectively). In conclusion, ET-1 plays some role in the development of retinopathy in diabetic patients in cooperation with the markers of inflammation and may be helpful to predict the presence of proliferative diabetic retinopathy in patients with diabetes mellitus. Thus identifying and treating markers for vascular disease in diabetes is significant in preventing long-term disabilities and containing health care costs, also it recommended to maintain normal blood glucose levels in diabetes patients, as blood glucose that is elevated for several years is a major factor in the development and progression of microvascular disease complications.

Key words: Type 2 diabetes, diabetic retinopathy, ET-1, IL-6, CRP, α-1 antitrypsin, haptoglobin

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INTRODUCTION

Diabetic retinopathy is the most frequent cause of adult blindness in patients 30-70% worldwide, which is a highly specific vascular complication of both type 1 and type 2 diabetes. The prevalence of retinopathy is strongly related to the duration of diabetes and the degree of metabolic control of patients. High blood glucose values lead to important changes in cellular metabolism and these alterations cause endothelial dysfunction that sets in motion morphological process of diabetic retinopathy. There is general agreement that early diagnosis and treatment of diabetic retinopathy can slow its progression and help to prevent blindness[1]. Reducing the incidence and slowing the progression of diabetes related microvascular complication remains a difficult challenge. In case of diabetic retinopathy, tight glucose and blood pressure control are difficult to achieve and maintain in practice, yet still only provide partial protection against sight threatening complications in the lifetime of a person with diabetes[2].

Angiogenesis is the major feature in the pathogenesis of Proliferative Diabetic Retinopathy (PDR). In this condition, retinal neo-vascularisation has a catastrophic effect on vision by causing vitreous hemorrhage, retinal detachment with formation of a fibro-vascular membrane and eventual blindness the factors that stimulate the growth of retinal blood vessels have not been fully defined but circumstantial evidence indicates that this not only involve angiogenic cytokines but also vasoactive hormones such as endothelin, which is a potent stimulator of cytokines, recent studies showed inhibitors of this vasoactive hormone pathway may confer organ protection in diabetes by inhibition of growth factors expression[3].

Endothelin-1 (ET-1) is one of the vasoactive factors synthesized and released by vascular endothelium, it is a potent paracrine vasoconstrictor peptide and it induces vasoconstriction and stimulates vascular smooth muscle growth. In the human eye, ET-1 like immunoreactivity has been documented in association with retinal blood vessels and ET-1 receptors have been found on both retinal vessels and pericytes[4]. Endothelial dysfunction causes an increase in inflammatory activity, possibly through the elaboration of pro-inflammatory cytokines, thus creating a vicious cycle of inflammatory activity and endothelial dysfunction[5].

Inflammation has been suggested as a risk factor for the development of microvascular complications in case of type 2 diabetes. As a marker of systemic inflammation C-reactive Protein (CRP) is principally produced in hepatocytes under the influence of cytokines especially IL-6 which induces the expression and release of CRP. Cytokines made by other tissue may induce the production of CRP in liver leading to elevated plasma levels. The elevated plasma levels of CRP are in part a marker of low-grade inflammatory state. Chronic inflammation emerges as a potential mediator of micro and macro-vascular diseases in diabetes[6].

The aim of this study was to examine whether ET-1 plays a role in the progression of diabetic retinopathy in cooperation with the other inflammatory markers and to determine the relationship between stages of diabetic retinopathy in patients with type 2 diabetes and inflammatory activity in these patients by measuring serum Il-6, CRP, haptoglobin and α-1 antitrypsin as markers of inflammation and to investigate their association with plasma ET-1 as a marker of endothelial dysfunction.

MATERIALS AND METHODS

This study was carried out on 55 patients suffering from non-insulin dependent diabetes mellitus (type 2 diabetes), diagnosed according World Health Organization (WHO) criteria, who presented to Retina Clinic of Ophthalmology of Research Institute of Ophthalmology seeking advice as regard eye complications of diabetes.

The best-corrected visual acuity was measured; slit lamp and fundus examination was performed. Those who were judged to have retinal affection were referred to do fluorescence angiography; finally patients were divided into three groups:

Group I: Including 15 diabetic patients with no retinopathy

Group II: Including 20 diabetic patients with Non-Proliferative Diabetic Retinopathy (NPDR) of varying severity.

Group III: Including 20 diabetic patients with Proliferative Diabetic Retinopathy (PDR) who received pan retinal photocoagulation either alone or together with pan planavittrectomy.

Beside 20 healthy, age and sex matched subjects were also included to serve as a control group.

Patients with ischaemic cardiovascular disorders, renal dysfunction and hepatic disorders were excluded from the study.

Sampling: Venous blood 10 mL was collected in the morning after 14 h fast from all subjects. Five milliliter

blood was put in a tube containing ethylenediamine tetra acetate "EDTA" as an anticoagulant to separate plasma from red blood cells after centrifuging for 10 min at 3000 rpm. The other 5 mL blood left to clot at room temperature to separate sera after centrifuging for 10 min at 3000 rpm. The plasma and sera were stored at -70°C until assay.

All patients were subjected to:

- Full history and full clinical examinations including personal history, history of diabetes (age of onset, duration and treatment)
- Laboratory investigations in the form of:

1. Determination of plasma endothelin-1 (ET-1) by use of quantitative enzyme immunoassay technique (ELISA) using kit supplied from (R and D systems, Minneapolis, MN). The ET-1 peptide was first extracted from plasma samples using a centrifugal evaporator after plasma-solvent dilution (water, HCL and acetone). An antibody specific for ET-1 has been pre-coated onto a microplate. Standard, samples, control and conjugate are pipette into the wells and any ET-1 present is sandwiched by the immobilized antibody and the enzyme-linked antibody specific for ET-1, following a wash to remove any unbound substances, substrate is added to the wells and color developed is proportion to the amount of ET-1 bound. The color development is stopped and the intensity is measured[7].
2. Determination of serum interleukin 6 (IL-6) by a solid phase enzyme amplified sensitivity immunoassay (EASIA) performed on micro titer plate using kit supplied by BioSource Europe S.A., Rue de industries, 8 B-1400 Nivelles Belgium[8].
3. Determination of serum C-reactive protein (CRP) by a high sensitive immunoassay for measuring human CRP which is a two step sandwich ELISA technique using kit supplied by Diagnostic systems laboratories (DSL-10-42100) Webster, Texas, USA[9].
4. Determination of serum α-1 antitrypsin by Bindarid radial immunodiffusion kit supplied by the Binding Site Ltd, Birmingham, B29 6AT, UK[10].
5. Determination of serum haptoglobin by Bindarid radial immunodiffusion kit supplied by the Binding Site Ltd, Birmingham, B29 6AT, UK[11].
6. Determination of serum creatinine by Jaffe reaction[12].
7. Determination of plasma glucose level by God-PAP enzymatic colorimetric method according to Trinder[13] using Biomerieux test kit, Cat. No. 5 127.1
8. Determination of serum aspartate (AST) and serum alanine transaminase (ALT) levels by using the method recommended by the committee on enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology[40], the test was performed using already commercially available kit from Boehringer-Mannhiem Company, German.

Statistical analysis: Descriptive statistics of the study population were expressed in the form of mean and standard deviation, comparison of the means of different groups according to the retinal affection was done using paired "t' test with statistical significance at $p<0.05$. The sensitivity, specificity, positive and negative predictive values were calculated for the cut off limit for ET-1, while the association of ET-1 and the inflammatory markers was calculated using Pearson product-moment correlation coefficient. All analyses were performed using Statistical Package for the Social Sciences (SPSS) software version 11.

RESULTS

This study was conducted on 55 diabetic patients type 2 presenting to Retina Clinic of Ophthalmology, Research Institute of Ophthalmology. The patients were divided into three groups according to the presence of retinal complication of diabetes.

Table 1 presents the demographic data of the patients and the control groups with no difference between these groups concerning age and the sex. All subjects had no hepatic or renal diseases as evident by normal ranges of liver enzymes (ALT and AST) and serum creatinine levels, also the fasting glucose levels and the duration of diabetes were presented in Table 1.

Table 2 shows the serum levels of ET-1 and the inflammatory markers (IL-6, CRP, haptoglobin and α-1 antitrypsin), the levels were expressed as range and mean±SD in the different studied groups.

Table 3 shows a comparative study of plasma ET-1 and the inflammatory markers levels in controls and the different studied groups. There were highly significant increased in the serum level of ET-1 in Group II and Group III of diabetic patients as compared to the control group ($p<0.001$), while Group I showed a non significant

Table 1: Clinical and biochemical features of the different studied groups

Parameters	Controls	Group I	Group II	Group III
Number	20	15	20	20
Sex (M/F)	11/9	6/9	9/11	12/8
Age (years)	52±4	51±6	55±7	58±5
Duration of diabetes (month)	-----	9.3±2.3	15.2±2.5	26.1±1.7
Fasting glucose (mg dL^{-1})	85±1.3	153±5.4	189±9.7	225±4.1
Serum creatinine (mg dL^{-1})	0.8±0.02	0.9±0.4	0.9±0.3	0.9±0.5
Liver function				
ALT (U L^{-1})	25.2±5.3	20.5±6.8	24.7±11.2	22.3±10.5
AST (U L^{-1})	24.6±5.3	23.2±8.8	26.9±9.8	22.9±8.5

Data expressed as mean±SD, M/F = male over female ratio. ALT = Alanine transaminase, AST = Aspartate transaminase, Group (I) patients with no diabetic retinopathy, Group (II) patients with non-proliferative diabetic retinopathy, Group (III) patients with proliferative diabetic retinopathy

Table 2: Statistical data of plasma ET-1 and inflammatory markers in the different studied groups

	ET-1 (pg mL^{-1})	IL-6 (pg mL^{-1})	CRP (mg L^{-1})	Haptoglobin (mg dL^{-1})	α-1 antitrypsin (mg dL^{-1})
Control group					
Range	0.66-1.52	1.01-1.96	0.35-2.61	89.5-160.75	103.00-213
Mean±SD	1.15±0.23	1.25±0.31	1.17±0.56	160.75±59.19	136.46±36.37
Group I					
Range	1.06-2.41	1.02-2.61	0.68-2.66	89.5-226	103.00-215
Mean±SD	1.46±0.44	1.52±0.54	1.53±0.64	190.97±63.56	148.97±43.07
Group II					
Range	1.27-4.99	1.2-4.92	0.52-3.71	95.5-286	110.00-231
Mean±SD	2.69±0.98	2.54±1.11	2.21±0.76	206.87±53.56	159.67±42.52
Group III					
Range	1.63-8.9	2.66-19.72	1.18-5.99	104.61-321	121.76-356
Mean±SD	3.70±1.79	7.98±4.41	3.41±1.37	217.35±72.22	182.39±71.71

ET-1= Endothelin-1, IL-6= Interleukin-6, CRP= C reactive protein

Table 3: Statistical significance of ET-1 and the inflammatory markers between the different studied groups (p values)

	ET-1	IL-6	CRP	Haptoglobin	α-1 antitrypsin
C vs. GI	0.0616	0.0579	0.096	0.1624	0.378
C vs. GII	1.14E-08**	1.21E-05**	1.36E-5**	0.0416*	0.0419*
C vs. GIII	1.19E-09**	1.44E-07**	2.72E-10**	0.02201*	0.0028*
GI vs. GII	0.0003**	0.00289*	0.0063**	0.6749	0.254
GII vs. GIII	0.0028*	5.03E-06**	5.51E-05*	0.7120	0.0469*
GI vs. GIII	2.99E-06**	6.33E-06**	1.39E-07**	0.0466*	0.02085*

Non-significant ($p \geq 0.05$), * Significant ($p \leq 0.05$), ** Highly significant ($p \leq 0.001$)

Table 4: Statistical correlation coefficient (r) between the ET-1 and the other inflammatory markers in the different studied groups

	Group I	Group II	Group III
ET-1 and Haptoglobin	-0.26169	-0.21156	-0.24107
ET-1 and IL-6	0.245539	0.38996*	0.342728*
ET-1 and CRP	0.288749	0.622667*	0.356829*
ET-1 and α-1 antitrypsin	-0.22172	0.026475	0.119645
ET-1 and glucose	0.02358	0.3505*	0.57856*
ET-1 and duration of diabetes	0.05326	0.32068*	0.35768*

* Significant ($p \leq 0.05$) positive correlation

Table 5: the sensitivity and the specificity of ET-1 (pg mL^{-1}) levels in the different studied groups

	Cut off value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Group I	1.32*	80.00	90	90.9	82.60
	1.33	73.30	95		
Group II	1.42	94.73	95	100.0	95.24
	1.51*	95.00	100		
Group III	1.63*	100.00	100	100.0	100.00
	2.06	95.00	100		

PPV= positive predictive value, NPV= negative predictive value. *Cut off level which maximizes the sum of sensitivity and specificity

increased in the serum level of ET-1 ($p>0.05$). There were significant increased in the serum level of ET-1 between the three studied groups (serum levels of ET-1 were high in Group III more than in Group II and I). The serum level of IL-6 showed a significant increased in Group II and III as compared to the control group ($p<0.05$), while Group I showed a non-significant increased in the serum level of IL-6 ($p>0.05$). There were a significant increased in the serum levels of IL-6 between the three studied groups.

The serum level of CRP showed a highly significant increased in Groups II and III as compared to the control

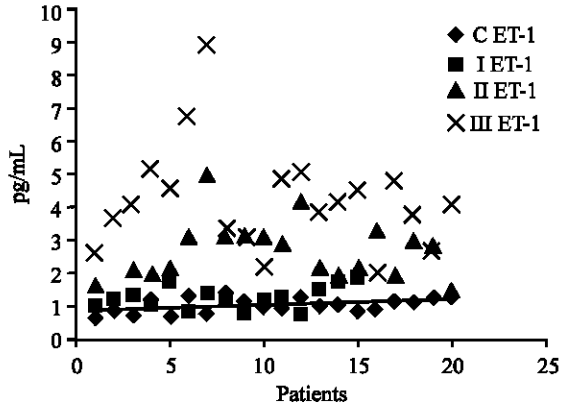

Fig. 1: Scatter presentation of plasma ET-1 (pg mL^{-1}) in the different studied groups

groups ($p<0.001$), while Group I showed a non significant increased in the serum level of CRP ($p>0.05$). There were significant increased in the serum levels of CRP between the three studied groups ($p<0.05$ and $p<0.001$).

The serum levels of haptoglobin showed significant increased in Groups II and III as compared to the control group ($p<0.05$), while Group I showed a non significant increased in the serum level of haptoglobin ($p>0.05$). There was significant increased in the serum level of haptoglobin in-Group III as compared to Group I ($p<0.05$).

The serum levels of α-1 antitrypsin showed significant increased in Groups II and III as compared to the control group ($p<0.05$), while Group I showed a non significant increased in the serum level of haptoglobin ($p>0.05$). There were significant increased in the serum level of α-1 antitrypsin in-Group III as compared to Group II and I ($p<0.05$).

Table 4 and Fig. 1 shows the correlation of plasma ET-1 with the other inflammatory markers (IL-6, CRP, haptoglobin and α-1 antitrypsin) in the different studied groups. There were statistically significant positive correlation ($p<0.05$) between ET-1 and both of the IL-6 and CRP($r = 0.389$ and 0.623, respectively). Also there were statistically significant positive correlation ($p<0.05$) between the plasma level of ET-1 and both of the glucose level and the duration of diabetes in Group II and III ($r = 0.3505$, 0.578 and 0.321, 0.357, respectively).

Table 5 showed the best cut off values of ET-1 in Group I was 1.32 pg mL^{-1} with sensitivity 80% and specificity of 90%, while in Group II the best cut off value was 1.51 pg mL^{-1} with sensitivity 95% and specificity of 100%, and in Group III the best cut off value was 1.63 pg mL^{-1} with sensitivity 100% and specificity of 100%. The positive and the negative predictive values in the three studied groups were (90.9 and 82.6%, 100 and 95.24% and 100 and 100%, respectively).

DISCUSSION

Understanding the causes of diabetic vascular complications has become an increasingly important issue because of the rapidly rising prevalence of diabetes. Recently discovered vasoconstrictors and angiogenesis regulators, such as endothelin (ET) and Vascular Endothelial Growth Factor (VEGF), have been intensely studied for possible pathogenic roles in diabetic vascular complications[14].

Diabetic retinopathy is a multifactorial complication in which glycometabolic factor plays an important but not an exclusive part, there is a growing evidence that retinopathy is not related to hyperglycemia and diabetic duration only but other risk factors have been shown to play an important role as well[1].

Hyperglycemia has been identified as a major risk factor for this complication, there is a great deal of evidence to support the conclusion that hyperglycemia is responsible for many early retinal capillary dysfunctions and lesions as micro aneurysms, basement membrane thickening, increases permeability and alteration of retinal blood flow[15].

In the present study, plasma endothelin-1 showed significant stepwise increase in patients with NPDR (Group II) and PDR (Group III) but not in patients without retinopathy (Group I). These results were in agreement with[16-19]. They stated that diabetic patients with retinopathy showed impaired endothelium-dependant vasodilatation and elevated endothelin-1 values. They reported that, retinal capillary closure, a hallmark of diabetic retinopathy, leads to tissue hypoxia, ischemia and neovascularisation of the retina develops adjacent to these areas of non-perfusion. They suggest that ET-1 could play significant role in disease course leading to characteristic changes in retinal and iris neovascularisation. ET-1 was significantly increased in patients with proliferative diabetic retinopathy as compared to controls.

ET-1 may also be involved in later stages of diabetic retinopathy; indeed this peptide is angiogenic for vascular endothelial cells and may regulate endothelial proliferation during retinal neovascularization[20].

Calles-Escandon and Cipolla[21] stated that because blood vessel formation is a complex, multistep process, there may be several angiogenic factors, each of which operates under different circumstances, or the various factors may work together synergistically. Endothelin-1, which is an endothelium derived vasotropic cytokine that can bind to three types of endothelial receptors, can lead to retinal vasoconstriction and decreased blood flow, which contribute to the establishment of pathogenesis in case of diabetes.

Laurenti *et al.*[22] suggested that ET-1 represent a marker of diabetes-related vascular damage. They stated that hyperglycemia triggers a cascade of biochemical events that lead to vascular dysfunction and early structural changes in blood vessel walls, also, vascular damage and endothelial dysfunction occur early in the course of diabetic microangiopathy leading to increased endothelin-1 levels which is vasoconstrictive peptide secreted by endothelial cells and decreased concentrations of endothelium derived relaxing factors and fibrinolytic potential of vascular endothelia of the retina.

In addition, ET-1 has been shown to promote retinal pericytes proliferation in a dose dependent manner. The combination of local paracrine effect of ET-1, along with the release of nitric oxide and other vasoactive factors, is likely to play a relevant physiological role in the regulation of retinal blood flow, especially because of the absence of autonomic innervations of retinal vessels[4]. Many retinal changes in diabetes are associated with changes in ET-1 levels. Elevated levels of ET-1 have been reported to decrease retinal blood flow and loss of auto regulation in response to O_2 tension in retina[23]. Experimental evidence suggest that ET-1 contribute to ocular pathological manifestations, promoting retinal capillary non-perfusion and ischemia, the biochemical mechanism that are responsible for the increased expression of ET-1 in the retina is initiated by hyperglycemia or glucose metabolites. Among various metabolites that are derived from hyperglycemia, increased levels of glaciation products, oxidants and the activation of signaling pathways such as Protein Kinas C (PKC) have been reported to increase expression of ET-1 in cultured vascular cells[24].

In this study there were significant positive correlation between glucose level and ET-1 in Non Proliferative Diabetic Retinopathy (NPDR) and Proliferative Diabetic Retinopathy (PDR) group, also there was significant positive correlation between duration of diabetes and ET-1 in both NPDR and PDR groups. The prevalence of retinopathy is strongly related to the duration of diabetes. After 20 years of diabetes, nearly all patients with type 1[25] and more than 60% of type 2 Diabetes have some degree of retinopathy[26].

These results were in agreement with Irving *et al.*[27] who showed that plasma ET-1 levels correlated with several risk factors as the duration of diabetes and hyperglycemia. Park *et al.*[28] reported that hyperglycemia is the most likely metabolic change inducing elevation of ET-1 in retina because the exposure of pericytes to elevated glucose levels can increase expression of ET-1 mRNA and protein level through increasing the PKC isoforms activity, suggesting that inhibition of these PKC isoforms may prevent early changes in diabetic retinopathy.

Yokota *et al.*[29] reported in their study for the first time that diabetes can increase Platelet Derived Growth Factor-B (PDGF-B) levels in retina, which could potentially be the initiator for the increase in ET-1 expression in retina. It is also likely that PKC activation induced by hyperglycemia could be regulating the increase of PDGF-B expression and its actions on ET-1 expression.

As regard to the markers of inflammations (IL-6, CRP, haptoglobin and the α-1 antitrypsin) results showed statistically significant increase in case of Non Proliferative Diabetic Retinopathy (NPDR) (Group II) and Proliferative Diabetic Retinopathy (PDR) (Group III) when compared to control group and the patients without retinopathy (Group I).

These results were in agreement with Stehouwer *et al.*[30] and Jagger *et al.*[31] who stated that in individuals with type 2 diabetes, inflammatory activity is increased and is strongly associated with endothelial function and increased incidence of complications. Rema *et al.*[32] studied the acute phase serum proteins in diabetic retinopathy, they reported that haptoglobin was elevated in diabetes with retinopathy and the level were higher with proliferative diabetic retinopathy, they stated that haptoglobin increases the serum viscosity and this could be the mechanism by which it plays a role in pathogenesis of diabetic retinopathy, while in contrast to the results of this study, they found no significant increase in α-1 antitrypsin in patients with retinopathy. Shimizu *et al.*[33] stated that IL-6 which is a pro-inflammatory cytokine is increased in case of diabetic retinopathy and can be an indicator of severity as it can predict diabetic macular edema Doganay *et al.*[34] reported that IL-6 which is a primary stimulant for hepatic acute phase response and is capable of inducing all acute phase proteins involved in inflammatory response, is increased in serum and vitreous, in case of proliferative diabetic retinopathy and they suggest that increased advanced glaciation end products in vitreous is involved in development of diabetic retinopathy by inducing the production of IL-6 from retinal Muller cells. Loukovaara *et al.*[35] and Pradhan *et al.*[36] stated that elevated glucose levels could promote inflammation by increasing oxidative stress and increasing the transcription factor nuclear factor kB, yet another possibility is that inflammatory response is a result of vascular complications following diabetes, as they reported that raised CRP, which is an acute phase reactant produced by hepatocytes and its chief inductor

is the cytokine IL-6, is associated with an increased incidence of microvascular complications and increased in case of diabetes with retinopathy with the highest values in proliferative diabetic retinopathy, this denoting that chronic inflammation emerges as a potential mediator of microvascular disease. Also, Knapik-Kordecka *et al.*[37] reported that α-1 antitrypsin activity is increased in patient with type 2 diabetes especially those suffering from microvascular complications and they showed significant correlation with glycemia control.

Present results showed a significant positive correlation between ET-1 and IL-6 in-Group II and Group III, also significant positive correlation between ET-1 and CRP in Group II and III.

The elevation of inflammatory markers is thought to be due, in part to hyperglycemia and the formation of advanced glaciation end products, which are a heterogeneous group of compounds that cause a number of adverse cellular events, including reduction of enzymatic activity, damage to nucleic acids, cross-linking and impaired degradation of proteins and induction of cytotoxic pathways[38,39]. Stehouwer *et al.* [30] have shown in a recent longitudinal study done on type 2 diabetic patients, that inflammation and endothelial dysfunction are mutually interrelated and progress with time, without one clearly preceding the other. This suggest that, in diabetes, inflammation induces endothelial dysfunction and that endothelial dysfunction increase inflammatory activity, thus creating a vicious circle, also the presence of microvascular complications influences the associations of conventional risk factors and endothelial function with inflammatory activity.

From the results of this study, it is possible to hypothesize that ET-1 and markers of inflammation play some role in the development of retinopathy in diabetic patients and could be considered reliable markers for this complication. Thus identifying and treating markers for vascular disease in diabetes is significant in preventing long-term disabilities and containing health care costs, also it recommended to maintain normal blood glucose levels in diabetes patients, as blood glucose that is elevated for several years is a major factor in the development and progression of microvascular disease complications. Further studies are needed to determine whether suppression of these risk factors by specific antibodies and anti-inflammatory agents, could prevent or improve proliferative diabetic retinopathy and its consequences.

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