Role of Angiopoietins and Tie-2 in Diabetic Retinopathy

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Abstract

Objective: The aim of this study was to determine the serum levels of angiopoietin-1 (Ang-1), angiopoietin-2 (Ang-2), soluble vascular endothelial tyrosine kinase receptor (Tie-2) and vascular endothelial growth factor (VEGF), in the serum of type 2 diabetic patients having non-proliferative (NPDR) or proliferative diabetic retinopathy (PDR).

Methods: One hundred patients with type 2 diabetes mellitus were involved in this cross-sectional study. They were classified into 25 diabetic patients without retinopathy, 35 patients with NPDR and 40 PDR patients. The study was carried out in the outpatients clinic of the Research Institute of Ophthalmology, Giza, Egypt from August 2016 through May 2017. Serum VEGF, Ang-1, Ang-2 and Tie-2 receptor levels were assayed using enzyme linked immunosorbent assay (ELISA). Data were analyzed by SPSS version 20 and Microsoft Excel (Version 10) using ANOVA, Harman's single factor test, and Pearson's Product Moment Correlation.

Results: The serum levels of Ang-2 and VEGF were significantly elevated in NPDR groups and PDR group compared to diabetics without retinopathy groups (p<0.001). The serum levels of Ang-1 were significantly higher in the NPDR group compared to the diabetics without retinopathy groups (p<0.01), while no significant difference was observed between the PDR and diabetics without retinopathy groups. Ang-1/Ang-2 ratio was the lowest in the PDR group compared to the NPDR and diabetics without retinopathy groups. The serum levels of Tie-2 were not significantly changed among the three studied groups, serum Ang-2 was positively correlated with VEGF and Tie-2 in the PDR and NPDR groups.

Conclusion: The angiopoietin /Tie system and VEGF are essential features in the commencement and development of PDR.

Keywords: Angiopoietins, Tie-2 receptor, Diabetic Retinopathy

1. Introduction

One of the most prevalent microvascular complications of diabetes is diabetic retinopathy (1). The initiation and progression of DR include various mechanisms such as: disorders in blood coagulation, oxidative stress and multiple neovascularization stimulating cytokines (2). Vascular endothelial growth factor (VEGF) is a key regulatory factor which participates in retinal and choroidal neovascularization and in excessive retinal vascular leakage (3). These types of microcirculatory disorder are the cause of retinal ischemia and the consequent upregulation of VEGF expression, and thus play an important role in the commencement of PDR (4). Angiopoietin are vascular growth factors involved in angiogenesis and vascular repair (5). Two major isoforms regulate vascular homeostasis, namely angiopoietin 1 (Ang-1) and angiopoietin 2 (Ang-2) (6). There are two other members of the angiopoietin family (Ang-3) and (Ang-4) (7). This Angiopoietin binds to the tyrosine kinase receptor with immunoglobulin and epidermal growth factor homology domain (Tie-2) on vascular endothelial cells (8). Tie-2 is a tyrosine kinase receptor predominantly existing on vascular endothelial cells which have a vital role in vascular stability. Ang-1 which are produced by perivascular cells, bind, and activate Tie-2, which lead to Tie-2, auto-phosphorylation and downstream signaling. The Tie-2 which is activated, increases survival of endothelial cells, adhesion and stability of

cell junction which results in stabilizing vasculature. While Ang-2 are negative regulators induced by ischemia. Ang-2 inactivate Tie-2, destabilizing the vasculature and increasing the response to VEGF (9). Ang-1 and Ang-2 binds to the Tie-2 receptor and induces its phosphorylation, which affects physiological and pathological angiogenesis by Ang/Tie pathway (9). Angiopoietin-1 and 2 are endogenous ligands for the vascular endothelial receptor tyrosine kinas. Angiopoietin -1 signals vascular endothelial survival and the reorganization of blood vessels and also inhibits leukocyte migration and leakage into tissues. Angiopoietin -2 generally has an opposing action to angiopoietin -1, and is released during vascular growth and in inflammation. The damage of the blood retinal barrier, which is induced by diabetes was inhibited by Ang-1 in a dose dependent manner (10). Accordingly, Ang -1 is protective against diabetic retinopathy, while it was found that Ang -2 induces vascular budding and increases vascular permeability, which leads to the pathological damage in retinopathy (11). They function in both an agonistic and antagonistic manner (12). The equilibrium between Ang-1 and Ang-2 and the signaling pathway induced by their binding to Tie-2, play a vital role in angiogenesis of the retina in diabetic retinopathy DR (13). The neovascularization marks the onset of PDR. Several studies have detected that the expression of Ang-2 and VEGF in the retina are unregulated in PDR. Ang-2 and VEGF work in a synergistic manner in promoting vascular permeability and stimulating retinal angiogenesis (13, 14). The aim of this study is to measure the serum levels of Ang-1, Ang-2, Tie-2 receptor and VEGF of diabetic patients with and without retinopathy, to investigate the role of the angiopoietin/Tie-2 system and VEGF in PDR.

2. Material and Methods
2.1. Research design and participants
This is a case-control study that included 100 type 2 diabetic patients whose ages ranged from 50 to 60 years. They were classified into 25 diabetic patients without retinopathy, 35 patients with NPDR and 40 patients with PDR, attending the outpatient clinic of the Research Institute of Ophthalmology, Giza, Egypt from August 2016 through May 2017. Exclusion criteria were liver or kidney dysfunction, cerebrovascular disease (including myocardial infection, cerebral infarction and peripheral artery disease, and uncontrolled hypertension. Also, any patients with other ocular conditions such as, cataract, glaucoma or uveitis were excluded from the study.

2.2. Examinations
Venous blood samples were collected on EDTA to measure HbA1 c, and the other part was centrifuged, and serum was separated and stored at - 80 °C until assayed. Full ophthalmological examination was performed and medical history was taken for each patient including measuring the best corrected visual acuity and intraocular pressure, slit lamp examination to determine the anterior chamber depth by the goniolens and the presence or absence of iris neovascularization. Additionally, indirect ophthalmoscopy and bio microscopy to evaluate the grade of vitreous proliferation and determine the presence and nature of macular edema, fundus fluorescein angiography to differentiate between non proliferative and proliferative retinopathy, and laboratory assays of serum VEGF, Ang-1, Ang-2 and Tie-2 by enzyme linked immunosorbent assay (ELISA) were also carried out.

2.3. Ethics of research:
Written informed consent was obtained from all patients in this study. This study was approved by the Ethics Committee of the Research Institute of Ophthalmology (Egypt).

2.4. Data analysis:
Data were analyzed using descriptive statistics, ANOVA, Harman's single factor test, and Pearson's Product Moment correlation (r). Microsoft Excel (Version 10) and IBM© SPSS© Statistics version 20 (IBM© Corp., Armonk, NY, USA) were used for data analysis. The degree of association between the variables was assessed using Pearson's Product. Moment correlation (r), where values of $p < 0.05$ were considered significant.

3. Results
Table 1, shows the patient's characteristics in the three studied groups including age, duration, FBS, HbA1c. The duration of diabetes was significantly longer in the PDR group compared to the NPDR and patients without retinopathy ($p<0.01$, $p<0.05$ respectively). Table 2, shows serum levels of Ang-1, Ang-2, VEGF and Tie-2. The levels of Ang-2 and VEGF were significantly higher in the PDR than in NPDR and diabetics without retinopathy ($p<0.01$). While Ang-1 was not significantly changed in the PDR compared to diabetics without retinopathy, it was significantly higher in the NPDR compared to patients without retinopathy ($p<0.05$). No significant differences were detected in serum Tie-2 levels among the three studied groups ($p>0.05$). The ratio of Ang-1/Ang-2 in PDR groups were significantly lowered compared to diabetics without retinopathy and NPDR group ($p<0.01$). However, it was

not significantly different between NPDR and diabetics without retinopathy ($p>0.05$). The Ang-1/Ang-2 ratio in the PDR group was significantly decreased compared to the diabetics without retinopathy group. Also, Ang-2 was positively correlated with VEGF in PDR and NPDR groups r=0.3, 0.32 respectively ($p<0.05$). In the NPDR group, Ang-2 was positively correlated with Tie-2 and r= 0.29 ($p<0.05$).

Table 1. Characteristics of Diabetic Patients Included in This Study.

Variable	Diabetic without retinopathy; n=25	NPDR; n=35	PDR; n=40	p-value
Age	52±12	53±9	55±10	NS
Duration	3±4.1	4±3.2	6±6.1	<0.01
FBS (mg/dl)	180±55	190±6.1	227±8.4	<0.01
HbA1 C	9.3±2.4	9.2±2.2	9.1±2.3	<0.05

p<0.05 significant, NS= not significant

Table 2. Serum Levels Ang-1, Ang-2, VEGF, Tie-2 and Ang-1/Ang-2 ratio Among the Three Studied Groups

Variable	Diabetic without retinopathy; n=25	NPDR; n=35	PDR; n=40	p-value
Ang-1 (ng/ml)	11.7±7.8	12.1±9.1	11.9±8.9	<0.01
Ang-2 (ng/ml)	2.7±1.4	4.4±2.51	6.6±4.2	<0.0001
Ang-1/Ang-2	2.9	2.7	1.5	<0.001
VEGF (pg/ml)	160±41	305±80	541±290	<0.0001
Tie-2 (ng/ml)	3.6±2.9	3.7±3.2	4.1±2.7	>0.05

p<0.05 significant

4. Discussion

Apoptosis, endothelial permeability and vascular inflammation play a vital role in diabetic retinopathy (15). Loss of pericytes and vasculopathy is followed by leukocyte adhesion and hyper permeability of the retinal microvasculature (16). Tissue ischemia leads to VEGF expression and angiogenesis leads to proliferative diabetic retinopathy (3). According to our findings, serum Ang-2 and VEGF levels were significantly higher in diabetic patients with NPDR and PDR compared to diabetic patients without retinopathy. Also, the levels of serum VEGF and Ang-2 were more elevated in the PDR group than in the NPDR group, suggesting that their levels are related to the progression of retinopathy. This is in agreement with many recent studies that showed that serum VEGF and Ang-2 are increased in diabetic retinopathy (18-20). Hackett et al. (14) found that Ang-2 can promote the initiation of new vessel formation in retinas as a response to ischemia. Also, Hammes et al. (21) postulated that the synergistic action of Ang-2 and VEGF promote hypoxia induced retinal angiogenesis, which is a pathological feature of PDR (20). Another study showed that Ang-2 can promote the neovascularization in the retina in response to hypoxia (21). Thus, Ang-2 is associated with pathological angiogenesis in patients with PDR (21). This study detected that Ang-2 and VEGF levels were significantly correlated with Tie-2 levels. We suggested that the Ang-2/Tie signaling system may interact with VEGF during the initiation of DR. Microvascular disorders lead to retinal ischemia, which increases VEGF expression. VEGF and hypoxia upregulate Ang-2 expression (22), Ang-2 interacts with VEGF to promote neovascularization (23). In the absence of VEGF, Ang-2 suppresses the ability of Ang-1 to phosphorylate the Tie-2 receptor causing vascular damage and endothelial cell apoptosis (24). Serum levels of Ang-2 and VEGF were significantly correlated with serum Tie-2 levels, suggesting that the Ang/Tie signaling may react with VEGF during the initiation of diabetic retinopathy (15). Microvascular disorders bring about retinal ischemia which upregulates VEGF expression. At the same time, VEGF and ischemia can upregulate the expression of Ang-2 (18, 19). Subsequently, VEGF and Ang-2 appear to interact together to encourage angiogenesis (11, 12). Several studies have detected that the effect of Ang-2 on vessels is dependent on the presence of VEGF (11, 12, 17). This study showed that the serum Ang-1 level was significantly elevated in patients with NPDR compared to those without diabetic retinopathy and PDR patients. It was suggested that serum Ang-1 levels increase early in diabetic retinopathy as a compensatory mechanism to help cellular repair and preserve the integrity of the endothelial cell (25). Thus, promoting the stabilization and maturation of new micro vessels and the integrity of the micro vascular structure (26, 27). As Ang-1 and Ang-2 compete with each other for the Tie-2 receptor and have opposite functions concerning neovascularization, it is not sufficient to measure only one level of angiopoietins (28). So, to assess the equilibrium of ongoing angiopoietin process, we determined the ratio between the two antagonistic angiopoietins. The ratio between Ang-1 and Ang-2 decreased in the NPDR stage suggesting that the equilibrium between these angiopoietins have been changed in the early period of DR. In the PDR group, the ratio was almost half that in the NPDR group. Our results are in agreement with You et al. (28), who suggested that with the progression of DR, an

increased level of Ang-2 and marked changes levels of Ang-1, shifts the equilibrium towards neovascularization. This Study detected that Tie-2 levels were not significantly different among three studied groups of patients. These results revealed that Tie-2 levels may not be associated with the progression of DR (29). The presence of Ang-2 and VEGF, suppresses the effect of Ang-1in a competitive manner, thus breaching the endothelial cells' connection and consequently, leading to increased permeability of the vessel, inducing hypoxia-related retinal angiogenesis (30, 31).

5. Conclusions
In summary, this study detected that serum Ang-2 and VEGF are associated with the progression to PDR in type 2 diabetics. Ang/Tie system and VEGF, play an important role in the initiation and aggravation of PDR. Therefore, increasing Ang-1/Ang-2 ratio can help vessel integrity which may represent a new therapeutic target for treatment of diabetic retinopathy. Also, the Ang-1/Ang-2 ratios which influence the vascular integrity are still required to be established.

Conflict of Interest:
There is no conflict of interest to be declared.

Authors' contributions:
All authors contributed to this project and article equally. All authors read and approved the final manuscript.

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