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Effect of sunitinib in treatment of retinal angiogenesis induced by VEGF165 in rabbit's eyes

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Abstract

Objective:
The present study was designed to investigate the effect of sunitinib in treatment of retinal angiogenesis which is induced by vascular endothelial growth factor-165 (VEGF-165) in eyes of rabbits. Sunitinib is multi-tyrosin kinases receptor inhibitor which used in treatment of particular types of tumors and has effects on serum level of matrixmetalloproteinase-9 (MMP-9) and intercellular adhesion molecule-1 (ICAM-1) that are important mediators of angiogenesis.

Materials and methods:
Twenty four Dutch-belted rabbits weighing (1.3-2.7 Kg) are involved in this study. They were divided into four group (each with 6 rabbits and all were injected to their right eyes through intravitreal injection) as following:

- Normal group : they were received 0.1ml of sterile phosphate buffer saline (PBS)
- Angiogenic group: they were received 0.1ml of VEGF-165
- Ranibizumab-treated group: they were received 0.05ml of ranibizumab
- Sunitinib-treated group : they were received 0.1ml of sunitinib malate .

Results:
Intravitreal injection of VEGF-165 in posterior jumper of the eyes rabbits cause significant increase in (ICAM-1) and (MMP-9) associated with extensive retinal angiogenesis. Administration of sunitinib malate solution and ranibizumab cause significant decrease in ICAM-1 and MMP-9 associated with complete recovery from angiogenesis compared with angiogenic group. PBS treated group associated with normal retina without agiogenesis as indicated with non significant increase in ICAM-1 and MMP-9

Conclusion :
From this current study we can conclude that sunitinib can be added to anti VEGF drugs that are used for treating retinal angiogenic diseases such as proliferative diabetic retinopathy and diabetic macular oedema, the major cause of blindness.

Keywords: retinal angiogenesis, VEGF, sunitinib, intercellular adhesion molecule-1, matrixmetalloproteinase-9.

INTRODUCTION

Eye Structure :

The eye is the most sensory and complex structure in the body which responsible for sight. It composed of three parts through them the eye do its function, the outer part consist of cornea and sclera; the cornea is a stronger tissue that refract and transmit the ligt through the lens to the retina and protects the deeper parts of the eyes against infection and structural damage. The sclera is a coat of connective tissue, which protects the eyes from the internal and external forces and maintains its shape; the visible part of the sclera is the conjunctiva which is a transparent mucous membrane. The middle part of the eye include the iris, the ciliary body and the choroid. The iris regulate the size of the pupil; the ciliary body account for production of aqueous humor and maintain shape of lens and its power ; the choroid is a vascular layer which provides nutrients and oxygen to the retina. [1]

The inner part of the eye is the retina which is light sensitive tissue consist of deferent types of neurons that transform images into neuronal signals in order to sent them to the visual cortex through optic nerve for managing.[2] These neurons are amacrine cells, ganglion cells, , horizontal cells, bipolar cells and photoreceptors (cone and rod) that arranged in multiple parallel layers.[1] Retinal pigment epithelial (RPE) cells is the outer layer of the retina which separate photoreceptors from choriocapillaries. These cells are metabolic sensor that resbonsible for production prangiogenic factors that effect vascular tone and function of photoreceptors.[2] The retina is supplied by two blood supplier: the central artery of the retina and the choriocapillaris. Retinal blood vessels supply oxygenated blood up to the inner two third of neuronal retinal layer whereas the choriocapillaris supply the photoreceptor layer ,which is avascular layer, with their need of oxygen by diffusion.[3]

The aqueous humor is a colorless fluid which fill the area between the cornea and the lens and supplies nutrition, transfers neurotransmitters, removes west products resulted from metabolism, supports structure of the eyes and maintains the homeostasis of the ocular tissues. permits the inflammatory cells and mediators to distribute within the eye during pathological conditions, and permits drugs to circulate to various ocular tissues.[4] The vitreous humor is a transparent, gel-like fluid, located between the lens and the retina. It maintains the retina in place and keeps the globular shape of the eyeball.[5]

Neovascularization:

Neovascularization is controlled process of new blood vessels (BV) generation which involve two main mechanism that are [6] vasculogenesis ,the process of blood vessels development during embryonic growth ,firstly Mesoderm cells first differentiate into endothelial precursors cells (EPC) (angioblasts) and then into endothelial cells(EC) that unit togethers to form primitive tubes that then expand. Subsequent blood vessel formation take place by angiogenesis .[7] There are two types of angiogenesis sprouting and non – sprouting angiogenesis;

sprouting angiogenesis involve steps of antigenic stimuli, Sprouting, Elongation and branching, Tubulogenesis , lumen formation, and Anastomosis,Stabilization/regression .[8, 9] non sprouting or intussuseptive angiogenesis characterized by the presence of specific structural feature known as intussusceptive pillar ,a cylindrical microstructure that cross the lumen of small vessels and capillaries.[10] It faster than sprouting angiogenesis , nither involves degredation of basement membrane nor endothelial proliferation and migration. Rather it includes hypertrophia of ECs that then become flatten.so that it considered less metabolic energy consuming than sprouting angiogenesis.[11]

Regulation of angiogenesis:

Angiogenesis is regulated by balance between proangiogenic factors (such as acidic fibroplast growth factor (aFGF) , basic fibroblast growth factor(bFGF) and vascular endothelial growth factors (VEGFs)) and antiangiogenic growth factors such as (angiostatin,endostatin).[12]

The major angiogenic promoter in physiological and pathological conditions is VEGF. VEGF are family of cytokines which include VEGF-A orVEGF,VEGF-B,VEGF-C,VEGF-D and placental growth factor (PlGF). They involve in the process of vasculogenesis, angiogenesis and lymphangiogenesis. VEGF-A is a 45 kilo dalton heterodimeric heparin-binding protein. Alternative splicing of VEGF gene generates four isoforms, VEGF165,the predominant one,VEGF121, VEGF189, and VEGF206. VEGF also known vascular permeability factor (VPF), Stimulates endothelial cells growth , proliferation and vascular permeability, preserves the survival of endothelial cells (EC) and prevents their apoptosis.[13] VEGF-A binds to tyrosin kinase receptors(RTK)that are VEGFR-1 and VEGFR-2 and non- RTK that are neuropilin (NRP) receptors family(NRP‑1 and NRP‑2) as co-receptors.[14]

Retinal angiogenic diseases:

The imbalance between proangiogenic and anti angiogenic factors results in development of abnormal, new blood vessels within the retina forming retinal angiogenesis .Retinal angiogenesis is a pathological condition presented in several ocular diseases such as diabetic retinopathy (DR), retinal vein occlusion (RVO) and retinopathy of prematurity (ROP) that lead to loss of vision.[15]

During retinal ischemia, Retinal pigment epithelium and all main types of retinal neurons will release pro angiogenic factor, mainly VEGF ,in response to hypoxia. VEGF will induce neovascularization and inflammation by stimulating the expression of adhesion molecules. In addition, inflammation potentiates retinal neovascularization through production of multiples cytokines that either act directly on endothelial cells or indirectly through induction of leukocytes and or endothelial cells to form angiogenic factors.[16] The new ,developed vessels result in sever vision problems because of hemorrhage, fibrosis, tractional retinal detachment and vascular leakage that cause retinal edema.[17]

Diabetic retinopathy:

Diabetic Retinopathy is the most popular, chronic, microvascular complication of Diabetes Mellitus which lead to vision loss among working peoples in developed countries.DR prevalence associated with duration of diabetes and the level of glycemic control in addition to other risk factors that include hypertension, dyslipidemia and nephropathy.[18]

DR occurs when there is sustained , chronic elevation of blood glucose level cause accumulation of glucose in the endothelial cells (ECs) of retinal microvessels .This results in activation of several biochemical pathways include oxidative stress ,Aldose reductase and polyol pathway, Advanced glycation end products (AGE), activation of Protein kinase C, activation of Mitogen-activated protein kinase, Leukostasis and platelet activation ,Nuclear factor-kappa B (NF-κB), Inducible nitric oxide synthase Cyclo-oxygenases, Intracellular cell adhesion molecules ,Vascular endothelium growth factor , Interleukins-1-beta,andTumor necrotic factor-alpha.[19] finally,they lead to functional and structural alterations in the microvesseles and neuroglial parts of the retina include thickening of capillary basement membrane (BM), loss of pericyte and endothelial cell , breakdown of blood-retinal barrier (BRB) and leakage, acellular capillaries, and neovascularization.[20]

DR are classified into two different stages: non proliferative DR (NPDR); it occurs at the beginning of DR when retinal blood vessels become weakened ,damaged and leaking fluid in to the retina lead to retinal swallowing result in hemorrhages (HMs), microaneurysms (Mas), exudates (EXs), and interretinal microvascular abnormalities (IRMA). Prolifertive diaberic retinopathy (PDR) is the progressive stage of DR ,characterized by developing a new blood vessels in the retina and induced by retinal ischemia as a result of The microvascular changes that occur in NPDR. [20]

Diabetic macular edema (DME) is the thickening of retina that occurs in the center of macula. It considered the major cause of blindness among diabetic patients DME can take place at any stage of DR as a result of retinal hypoxia which increase expression of (VEGF),VEGF in turn induce vascular permeability and the trigger the formation of abnormal and leaking new vessels ,increase release of inflammatory cytokines and lack the tight junction among endothelial cells causing damage of blood- retinal barrier and thus accumulation of fluid in different area in the retina. [21]

Retinopathy of prematurity (ROP)

Is an ocular disorder which effect the retina of preterm, infant of low birth weight causing preventable vision loss at the childhood due to development of fibrous tissue behind the lens. It involves two phases, vaso-obliterative phase ;which begins immediately at birth to 30-32 week postmenstual age (PMA)and characterized by the retardation of normal growth of retina due to sudden reduction in insulin-like growth factor-1 (IGF-1) and VEGF. When the infant matures, the avascularized retina becomes greatly metabolically active, results in tissue hypoxia and thus development of subsequent phase if left without interruption. Vasoproliferative Phase of ROP takes

place at 32–34 weeks of PMA ,this phase characterized by increased hypoxia of the a vascularized retina that causes over production and releasing of VEGF and angiogenesis. The neovascularization and its subsequent fibrosis will cause retinal detachment and finally vision loss unless adequate oxygen therapy is administered.[22]

Retinal vein occlusions (RVOs):

Is a retinal vascular disorders, characterized by engorgement and dilatation of retinal veins due to increased venous blood pressure of the retina causing hemorrhages in the retina and subretinal space, macular edema, and retinal ischemia. RVOs are divided in to two types: central retinal vein occlusion (CRVO) ;which caused by increasing intraocular pressure, an atherosclerotic central retinal artery, and deformation of the lamina. Branch retinal vein occlusion (BRVO) results from venous compression of the vein at the arteriovenous (A/V) crossing, vessel wall degeneration and abnormality in hematological factors.[23, 24]

Diagnosis of Retinal angiogenic diseases:

Fundus imaging is a primary method through which 3 dimentional retinal structure is designed to the 2 dimension into imaging plane by using reflected light. Fundus imaging characterized by its safety and cost –effectiveness in documented retinal abnormalities .various retinal fundus photograph are used for diagnosis retinal structural abnormalities in different diseases like fundus photography, color fundus photography, and fluorescence angiography[25] , Binocular indirect ophthalmoscopy (BIO) or wide-field fundus imaging, telemedicine (TM)-based remote digital fundus imaging. Optical coherence tomography (OCT)[26, 27] , concentration of different molecules changed during ocular diseases like diabetic retinopathy to induce retinal angiogenesis for example increase concentration of (MMP-9) ,(ICAM-1) and other inflammatory and growth cytokines. [28]

Matrix metalloproteinases (MMPs):

Are groups of endopeptidases that degrade the connective tissues. MMPs have important roles in many physiological and pathological conditions like wound healing immunity, development of the embryo , tumor invasion and metastasis ,angiogenesis and inflammation.[29] Most of the MMPs are inhibited by specific endogenous tissue inhibitor known as tissue inhibitors of matrix metalloproteinase [TIMPs].

Among MMPs, MMP-9, also called gelatinase B, play important role in agiogenic process. MMP-9 breakdown ingredients of the extracellular matrix, encourage tissue remodeling and activate growth factors, like vascular endothelial growth factor-A (VEGF-A). MMPs and VEGF appeared to produce seemingly unilateral effects on each other in term of their expression and production.VEGF can regulate production of specific MMPs in stromal cells as well as in activated endothelial cells. therefore, MMPs act both upstream and downstream of VEGF.[30]

Inter cellular adhesion molecule-1(ICAM-1):

Is a membrane-bound glycoprotein . it acts by stimulating leukocyte trafficking and it is released from different cell types such as endothelial cells, epithelial cells, leukocytes and others. Under normal condition ICAM-1 expression is low compared with higher expression during inflammation stimulated by different cytokines.[31] VEGF stimulate inflammatory response in the retina through increase expression of ICAM-1. ICAM-1 increased adhesion of leukocytes to vascular endothelium of the retina will result in endothelial damage , collapse of the blood retina barrier, nonperfused capillary ,and ischemia lead to neovascularization. [32] In addition to leukocytes activation , ICAM-1 stimulate cytokine production which mediate inflammatory reaction and increase release of VEGF.[33]

Treatment of retinal angiogenic diseases :

Laser photocoagulation the standard treatment used to decreases or reverses retinal neovascularization which produce thermal burning in retinal tissues that produce growth factors.[34] Vitrectomy is a surgical procedure that involve removal of vitreous darkness or fibrovascular proliferation. It is indicated for vitreous hemorrhage and tractional retinal detachment.[35]

Anti angiogenic drugs:

VEGF considered the main factor that stimulate angiogenesis in ocular diseases. so that, VEGF is the main target for drugs used to treat pathological angiogenesis. Anti angiogenic drugs act by three main mechanisms:[36]

1. Drugs bind with VEGF directly for example, Pegaptanib aptmer (Macugen), Bevacizumab (Avastin), Ranibizumab (Lucentis), Aflibercept (Eylea).
2. Drugs act by inhibition of VEGF synthesis: by using silencing RNA (siRNA) sequences, a double-stranded RNA that are capable of silence the VEGF gene due to mutual homology. siRNA sequences penetrate across cellular membranes, block post-transcriptional RNA process, and thus inhibit synthesis of VEGF.
3. Drugs act by inhibiting VEGF signaling; through blocking RTK for all members of VEGF family for example , pazopanib and multi-kinase inhibitors, like sorafenib and sunitinib that are used for treatment of renal and hepatocellular cancer, and there are several trails to use them in treatment of ocular neovascular diseases.

Sunitinib:

Chemically, sunitinib is indolin-2-one analogs. is one of tyrosine kinase inhibitors (TKI) that exerts its antiangiogenic and antitumor effects by blocking multiple receptor tyrosine kinases, like VEGF, PDGF receptors, colony stimulating factor receptor type 1 (CSF-1R),stem cell factor receptor (KIT), glial cell-line derived neurotrophic factor receptor (RET) and Fms-Like Tyrosine Kinase-3 (FLT3). Sunitinib was approved by FDA in 2006 as a drug for the treatment of metastatic renal cell carcinoma and imatinib-resistant gastrointestinal stromal tumor (GIST).[37]

In this current, experimental study we use sunitinib malate in vivo to investigate its effect in treatment of retinal angiogenesis in rabbits eyes induced by VEGF-165.

MATERIALS AND METHODS

Recombinant human VEGF 165 (R and D system, USA), Human plasma albumin(CSL Behring AG ,Switzerland) ketamin (kepro, Holland), xylazine (kepro, Holland), Sodium dihydrogen phosphate powder (Riedel-Dehaen AG/Hannover,Germany), Sodium chloride powder (Sinopharm Co.Ltd. ,China) Di-sodium hydrogen phosphate powder (Fluka ,Switzerland), Ethyl alcohol 70% (Active cosmetics, China), Chloramphenicol eye drop (Amman pharmaceutical industries, Jordan), Ranibizumab 10mg/ml (Novartis ,France), Sunitinib malate powder (molekula, UK), Rabbit intercellular adhesion molecule1(ICAM-1) and Rabbit matrixmetalloproteinase 9(MMP-9) (Mybiosource -USA).

Methods:

Twenty four Dutch-belted rabbits weighing (1.3-2.7 Kg) are involved in the present study. They were handled according to the ethics committee in the College of Pharmacy/Mustansiriyah University. Rabbits were maintained in animal house of College of Pharmacy/Mustansiriyah University in iron cages ,each with four rabbits and kept healthy for three weeks under controlled condition of room temperature (21 ± 1^0C) and light stream of 12 hours light/ 12 hours dark cycles .they were received controlled pellets and fresh water. All rabbits were examined before beginning the study to ensure that they are normal on ophthalmic and general examination .Twenty four rabbits were divided in to four groups as illustrated in table 1.

Table 1: Animal grouping involved in the study

No. of group	Name of group	No. of rabbits	Description
1	Normal group(Negative control)	6 rabbits	They were received single dose of 10 unit(0.1 ml) of sterile PBS into posterior segment of the right eyes.
2	Retinal angiogenic group(Positive control)	6 rabbits	They were received single dose of 10 unit (0.1ml) of VEGF165 intravitrealy in the right eyes
3	Ranibizumab-treated group(Standard group)	6 rabbits	They were received 5unit (0.05ml) of ranibizumab as single dose, intravitreally in the right eyes after 1 week induction of retinal angiogenesis.
4	Sunitinib malate-treated group (test group)	6 rabbits	They were received 10 unit (0.1ml) of sunitinib malate solution as single dose intravitreally in the right eyes after 1 week induction of retinal angiogenesis.

Administration of VEGF-165 and induction of retinal angiogenesis:

Lypholized VEGF-165 was dissolved in 5ml solution of sterile, freshly prepared PBS and 0.1% human plasma albumin then stored at $2\text{-}8C^0$ until administration of solution of VEGF -165 within 7dayes as illustrated in data sheet from R and D system company.0.1ml of VEGF165 injected intravitreally to the right eyes of rabbit at 3.5–4.0 mm behind the limbus [38],[39], through specific syringe . After 1 week, the rabbits were sacrificed and vitreous fluid of the affected eyes were aspirated via 2ml sterile syringe stored in sterile ependorf at $-42c^0$ for measuring MMP-9 and ICAM-1 levels.

Administeration of sterile phosphate buffer saline

0.1ml of sterile PBS was injected in to right eyes of rabbits by intravitreal injection then after 1week ,the rabbits were sacrificed and vitreous samples were removed via 2ml sterile syringe and stored in sterile ependorf at $-42c^0$ untile measuring levels of MMP-9 and ICAM-1

Preparation and administeration of sunitinib malate:

Sunitinib malate solution prepared by dissolving 12.5mg of sunitinib malate powder into 1ml of sterile PBS that is compatible with ocular tissues and it shown not effect on drug stability. After 1week of induction of retinal angiogenesis , 0.1ml of freashly prepared sunitinib malate solution was injected intravitrealy.[40] The rabbits were kept for 29 days (similar to standard therapy) then they were sacrificed and vitreous humor obtained via specific syringe and putted in sterile ependorf at $-42c^0$ untile measuring the concentration of ICAM-1 and MMP-9.

Administration of standard treatment:

Rabbits were injected intravitreally by single dose of 5unit (0.5mg/0.05ml) of ranibizumab solution [41] .The rabbits were maintained for 29 day [42] then they sacrificed and the vitreous humor was removed from the affected eyes and stored in sterile ependorf at $-42c^0$ untile measuring levels of MMP-9 and ICAM-1.

Measurment of parameters: .

It include measuring of MMP-9 and ICAM-1 in vitreous sample by using enzyme –linked immunosorbent assay (ELISA) technology. ELISA technique is a method of quantitative analysis that used to measure even very low concentrations of substances like peptide ,vitamins and drug in biological fluid ,depending on antigen –antibody reaction through enzyme by using an enzyme-linked conjugate and enzyme substrate that observed by color changes.

Statistical analysis

In this study, data was analyzed using Statistical Packages for Social Sciences

(SPSS) software, version 16.Descriptive statistics were reported as mean± standard error of mean (SEM).Analysis of variance (ANOVA) test was used for verifying the significance of difference between the four studied groups, followed by Tukey test.

P value considered highly significant if it is < 0.01 and non significant if it is more than 0.05

RESULTS

Measurement of vitreous level of intercellular adhesion molecule -1

The mean ±SEM of ICAM-1 vitreous level was measured for all groups in this study. The statistical study showed that there was highly significant decrease ($P<0.01$) in mean concentration of ICAM-1 in vitreous humor in rabbit's eye of sunitinib (test)-treated group compared with positive (angiogenic) control group (0.15±0.02 ng/ml vs. 0.53±0.03ng/ml). The decreaed mean concentration of

ICAM-1 revealed no significant difference (P>0.05) between sunitinib-treated group and negative control group (0.15±0.02 ng/ml vs. 0.08±0.01ng/ml) and there was no significant difference (P>0.05)in decreased mean concentration of ICAM-1between sunitinib –treated group and ranibizumab –treated group (0.15±0.02 ng/ml vs. 0.08± 0.002ng/ml) .furthermore, the statistical study exhibit that there was highly significant decrease (P<0.01) in the mean concentration of ICAM-1 in vitreous humor between ranibizumab treated-group and angiogenic group (0.08± 0.002ng/ml vs. 0.53±0.03 ng/ml) also there was highly significant difference (P<0.01) in the mean concentration of ICAM-1 between control and angiogenic group (0.08±0.01ng/ml vs. 0.53±0.03 ng/ml) in addition there was no significant difference (P>0.05) in the mean concentration of ICAM-1 between control and ranibizumab-treated group(0.08±0.01ng/ml vs. 0.08± 0.002ng/ml) . Figure (3-1)

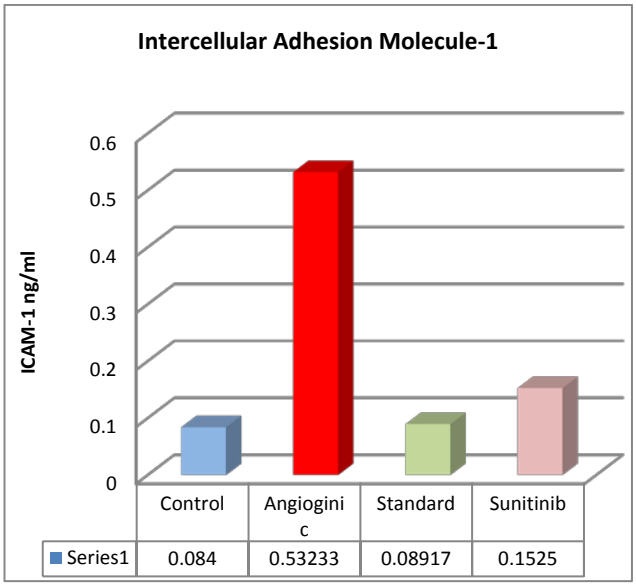

Figure (1): column chart of the mean values of ICAM-1level in vitreous humor of rabbit's eyes in tested groups. The results represented as mean± standard error of means (SEM).

Measurement of vitreous level of matrixmetalloproteinase-9

The mean ±SEM of MMP-9 vitreous level was measured for all groups in this study. The statistical study showed that there was highly significant decrease (P<0.01) in mean concentration of MMP-9 between sunitinib-treated group and angiogenic group (0.60±0.05ng/ml vs. 0.90±0.02 ng/ml) .also there was highly significant (P<0.01) difference in mean concentration of MMP-9 in vitreous of rabbits eyes between sunitinib-treated group and ranibizumab-treated group (0.60±0.05ng/ml vs.0.37±0.01 ng/ml) .Furthermore , statistics appeared that there was highly significant (P<0.01) difference in mean concentration of MMP-9 between sunitinib-treated group and control group(0.60±0.05ng/ml vs. 0.35±0.05ng/ml). moreover , there was non-significant (P>0.05) difference in mean concentration of MMP-9 between ranibizumab-treated group and control group (0.37±0.01 ng/ml vs. 0.35±0.05ng/ml) but there was highly significant (P<0.01) difference in mean concentration of MMP-9 between ranibizumab –treated group and angiogenic group (0.37±0.01 ng/ml vs. 0.90±0.02 ng/ml). Figure (3-2)

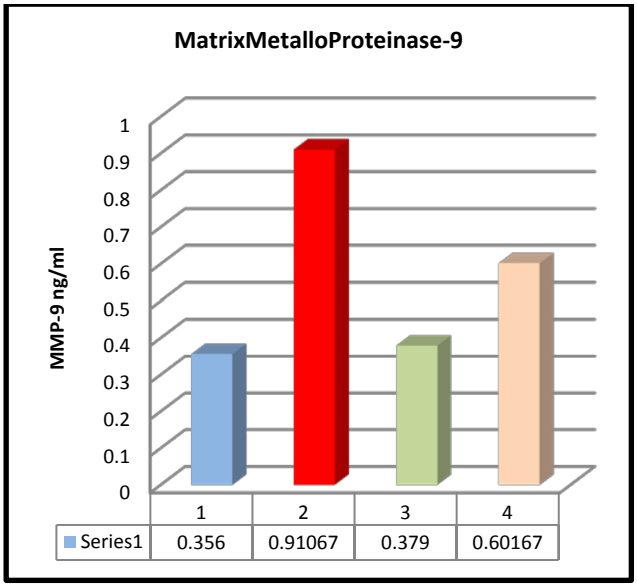

Figure (2): column chart of the mean values of MMP-9 level in vitreous humor of rabbits eyes in tested groups. The results represented as mean±SEM

DISCUSSION

Retinal angiogenesis is a pathological condition in the retina which associated with different retinal vascular diseases that lead to vision loss .The most important one among these disease is diabetic retinopathy.[43, 44] Proliferative diabetic retinopathy (PDR) and diabetic macular edema (DME) considered the major causes of visual impairment or visual loss in patients with diabetic retinopathy[45]. A major contributor to the development of abnormal blood vessels in PDR and macular swallowing is excessive production of VEGF[46] . It mediates its action through activation of VEGFR-1,-2[47] and stimulating different signaling pathways that result in generation of several mediators [48] that can be utilized as biomarkers to measure disease stages and evaluate responsiveness to appropriate therapy[49]

Induction of retinal angiogenesis

In this current study, recombinant human vascular endothelial growth factor 165 (VEGF165) was injected to the vitreous humor of right eyes of rabbits in order to form a model of PDR . after about 1week ,as mentioned in previous studies[38] ,the result appeared that there was a marked elevation in vitreous level of ICAM-1 and MMP-9 as indicators to development of retinal neovascularization (RNV).

Elevation of vitreous level of ICAM-1 in angiogenic group:

Several studies shown that the inflammation have an important role in pathogenesis of PDR [50-52]. inflammatory response induced by proangiogenic agent (VEGF) and it is represented by upregulation of ICAM-1

and other inflammatory molecules[53, 54] released by activated inflammatory and cells and glial cells that have a major role in the damage of retinal capillaries. Inflammatory cytokines stimulate adhesion of leukocytes to the endothelium , enhance vascular permeability, as well as thrombus formation by inducing imbalance between pro coagulant and anticoagulant activity[55] . leukocyte recruitment and adhesion to the retinal vasculature associated with capillary non perfusion and endothelial cell damage through releasing various inflammatory and growth cytokines that further enhance neovascularization [56].so that, there is agreement between previous studies that showed increase in ICAM-1 vitreous level in PDR in human and animal studies[57-59] and the current study.

Elevation of vitreous level of MMP-9 in angiogenic group

Extracellular matrix degradation has been involved in the pathogenesis of PDR. MMPs play an important role in ECM proteolysis which is an essential step for new vessels to get –away from the retina and inter the vitreous cavity [60]. In the present study, intravitreal injection of rhVEGF resulted in up regulation of MMP-9 which confirmed the generation of retinal angiogenesis and this agree with several studies[30, 61-63] that revealed that there is correlations between vitreous level of VEGF,MMP-9 and establishment of RNV.

Administration of sunitinib malate :

Angiogenic group was treated with single dose (10 unit) of sunitinib malate intravitreally, shown significant decrease in concentrations of measured biomarkers (ICAM-1 and MMP-9) in vitreous humor as a result of tyrosin kinase receptors (VEGFRs)blocking effect of sunitinib which inhibit downstream signaling of VEGF causing a reduction in RNV. This study considered a new research in studying the effect of sunitinib in treating RNV.

Administration of ranibizumab (standard therapy) :

In this study, administeration of ranibizumab as single intravitreal dose of 0.5 mg (0.05unit)[64] resulted in highly significant reduction in vitreous concentrations of ICAM-1 and MMP-9 with concomitant inhibition of RNV due to its action by neutralizing effect of VEGF and preventing VEGF from binding with its receptors and activating signaling pathways that lead to abnormal formation of blood vessels in the retina [65].This agree with what is revealed in various studies to show the therapeutic response of ranibizumab in treatment of PDR and DME [66, 67] .

CONCLUSION:

From this current study we can conclude that the sunitinib malate used in management of RNV model in experimental rabbits approximately as effective as standard therapy so that sunitinib malate can be added to anti-VEGF drugs used for treatment PDR and DME that are the major reasons for blindness in the world.

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