Biochemical Alterations in patients of diabetic retinopathy resulting from Type 2 Diabetic Mellitus-A Review

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Submitted: 01-08-2022

ABSTRACT

Diabetic Retinopathy (DR) is a potentially blinding complication of type 2 Diabetes Mellitus (DM). There are significant biochemical alterations in the course of the disease process which must be monitored. Increased blood glucose gives rise to increased expression of VEGF and its receptor VEGFR2. In addition, there is increased lactate, reduced NAD+, increased extracellular glutamate, increased reactive oxygen species (ROS) formation and nitric oxide formation. There is increased intracellular calcium, which activates endogenous phospholipase enzyme. This causes pericyte loss, vascular occlusion, capillary drop out, ischaemia, followed by increased VEGF and its receptor and neovascularisation. Retinal oedema may occur and impair vision if located in the central part of the macula. These include estimation of serum lactate, serum MDA, serum nitrite and nitrate, plasma glutamate, serum AGEs, ROS and VEGF and VEGFR2. All these may be estimated by various techniques such as spectrofluorimetry (Figure-1), flow cytometry(Figure-2), ELISA test and other biochemical tests. The various factors have been discussed and the role of each factor in the causation of DR has been defined. The estimation of biochemical changes are likely to indicate whether a patient is at risk of developing DR or not and appropriate action may be taken at the appropriate time.

Keywords: Diabetes Mellitus, Diabetic Retinopathy, Endothelial cells, Pericytes, Advanced glycation end products, Glutamate, Lactate, reactive oxygen species, Vascular Endothelial Growth Factor, Vascular endothelial growth factor receptor 2.

I. INTRODUCTION

Diabetes Mellitus is a disease that can affect many organ systems, particularly the eye, often with devastating results. The number of diabetic patients in India is estimated to increase to around 80 million, by the year 2030 (1). India may ultimately become the diabetes capital of the world.

Diabetic Retinopathy (DR) is the most common microvascular complication of Diabetes Mellitus (DM). (2) Despite recent advances in the diagnosis and treatment of DM, based on clinical observations and non invasive investigation, DR remains the leading cause of blindness in adults, both developed and developing countries. (3,4)

Many biochemical alterations take place in the retina of patients of DR resulting from type 2 DM. The excessive glucose load adversely affects the cells of the tissues. There is direct toxicity of glucose, as well as, many biochemical alterations in the pathways of glucose metabolism. All these changes lead to DR, and if not controlled adequately, to blindness.

II. PATHOGENESIS AND PATHOLOGY

The earliest microvascular dysfunction in these patients is evoked by hypoxia, because of capillary closure and non perfusion. The hypoxic retina adjacent to acellular capillaries releases vascular endothelial growth factor (VEGF) in an attempt to increase the vascularity of the ischaemic area.

Hypoxia and its sequelae can develop even without vascular occlusion, because of insidious apoptosis of vascular endothelial cells (EC) of inner retina due to persistent hyperglycaemia. Increased intracellular glucose in retinal tissue results in increased anaerobic glycolysis, polyol pathway, increased lipid peroxidation, advanced glycation end product (AGE) formation and increased tissue expression of VEGF. This leads to breakdown of inner blood retinal barrier.

There is hyperpermeability of retinal capillaries resulting from structural damage to the capillary wall. This structural damage occurs due to toxic effects of metabolites of anomalous glucose metabolism, lipid peroxidation and endothelial cell...
injury. Leukostasis occurs inside the blood vessels due to increased secretion of VEGF.

There is the appearance of microaneurysms due to hyper cellular saccular out pouching of capillary walls - either solitary or in clusters. Spillage of lipid rich material from the hyperpermeable capillaries accumulate in the inner and outer plexiform layers in the form of hard exudates. Intraretinal dot and blot haemorrhages appears as deep red dots and flame shaped haemorrhagewith wispy margins in the nerve fibre layer.

Leakage occurs from microaneurysms or hyperpermeable capillaries resulting in localized retinal thickening or diffuse macular oedema. The centre of macula may be involved causing visual impairment.

Thus microangiopathy in retinal capillary bed in patients of DM is the most frequent single cause of blindness in adult patients belonging to the age group 20-75 years. However, all patients of type 2 DM, inspite of having prolonged hyperglycaemia, do not develop DR. Some investigator opine that inhibition of glycolysis due to inhibition of glycolytic enzyme is responsible for developing DR. Others state that probably accelerated glycolysis exhausts the supply of oxidized cofactors, resulting in pseudohypoxia and anaerobic glycolysis.

There is formation of nicotinamide adenine dinucleotide positive (NAD\(^+\)), which leads to various biochemical derangements, leading ultimately to DR. So redox state alteration of the tissues compels enormous amount of glucose to enter into abnormal biochemical pathways and results in cellular apoptosis. This lead to excessive secretion of VEGF.

### III. BIOCHEMICAL INVESTIGATIONS

#### A. Serum lactic acid

This can be measured by the commercially available Lactate Kit (Randox-LC 2389, UK) (Figure-3), following lactate oxidase and peroxidase enzymatic method, (Burtis,1998). This is found to be elevated in patients of nonproliferative diabetic retinopathy (NPDR)(Figure-4) as compared to patients having diabetes with no retinopathy (DNR).(12)

#### B. Serum malondialdehyde (MDA)

The colorimetric method for quantitative analysis of serum MDA free of interference from sialicacids, as described by Satoh (13) is generally used.Serum MDA is found to be elevated in patients of NPDR as compared to patients of DNR. (12)

#### C. Serum nitrite and nitrate

This is done by Griess Reaction. Griess reagent, (G4410, Sigma USA) is used. This assay indirectly detects NO activity because it is notpossible to detect NO in physiological system, as it decays within seconds. NO is consumed in the reaction with oxygen species and transition metals, forming nitrate and nitrite. This technique involves enzymatic reduction of nitrate to nitrite and then derivatisation is done, with spectrophotometric detection of nitrite at 540 nm (14). Serum nitrate may be doubly elevated in NPDR as compared to DNR. (12)

#### D. Plasma Glutamate

This is measured by the enzyme linked spectrofluorimetric assay as described by Wang and Chen. Plasma glutamate levels are raised in NPDR as compare to DNR (12).

#### E. Serum total advanced glycation end products

This is done by comparing the AGE protein adduct content in the clinical samples with a standard curve. The measurement are done by enzyme linked immune sorbent assay (ELISA) using kits such as Cell biolabs (catalog no STA 317, San Diego,USA). The procedure of AGE estimation is that described byStitt (16). The serum total advanced glycation end products are raised in NPDR as compared to DNR (12).

#### F. Measurement of reactive oxygen species (ROS) from mononuclear cells

These cells are obtained from heparinized blood using Histopaque 1077 (from PBMC). ROS sensitive cell permeablendy 2’7’-dihydropidchlorofluorescein diacetate, in the presence of ROS is oxidised to highly fluorescent 2’7’-dichlorofluorescein in the cell. The fluorescence level is measured by flow cytometry and the ROS is directly proportional to the oxidation of the dye. The ROS generation is significantly more in NPDR as compare to DNR. (12)

#### G. Measurement of serum VEGF and VEGFR2

This is measured by using ELISA kit (Figure-5) available commercially (My Biosource, San Diego, CA, and Raybiotech Norcross, USA), according to the manufacturer’s instructions. Serum VEGF is almost double in NPDR patient while VEGFR2 levels are also increased in NPDR, both in comparison to DNR.
IV. DISCUSSION

The biochemical derangements are the causative factor for the development of DR, in patient of type 2 diabetes mellitus. DR develop in type 2 DM usually after 15 years of disease duration. It may develop early if the glucose control is poor. Again, DR develops earlier in patients of type 1 DM than type 2DM. Here also the onset of DR is earlier if the glucose control is poor. In case of type 2 DM, 60 percent of patients develop DR after 15 years, while 40 percent of patients remains asymptomatic. The main causal factor is chronic uncontrolled hyperglycaemia, which leads to the development of microangiopathy.

In the pathogenesis of DR, it has been found that the process of accumulation of AGEs, increased extracellular glutamate concentration, nitric acid secretion, lipid peroxidation and excessive generation of oxidative stress, all contribute to the increase tissue expression of VEGF and its receptor VEGFR2. Many studies have demonstrated the principal causal role of VEGF in pathogenesis of DR. However, as to how the biochemical derangement promote the increased tissue expression of VEGF and VEGFR2 is not known with certainty. (19,20)

VEGF also act as a neuroprotectant in ischaemic injury. (21) However, as pericytes are lost before the earliest manifestation of microangiopathic features, other mechanism may cause pericyte loss, in retinal capillaries (9). Hyperglycaemia by itself, is associated with accelerated death of vascular and neural cell of the retina. (22) Enormous quantities of glucose in the retinal cell results in faster glycolysis and polyl pathways. This results in NAD+ deficiency. In addition, sorbitol dehydrogenase and glyceraldehydes-3-phosphate dehydrogenase enzymes also consume NAD+ in large quantities, to remain active. (23)

This results in a condition of pseudohypoxia and there is anaerobic glycolysis. This causes excess lactate formation and cause the ratio of lactate to pyruvate to elevate, while the ratio of NAD+ /NADH falls (10). Again, excess lactate lowers the pH of the microenvironment of retina. This impairs the activity of L-glutamate/L-aspartate transporter (GLAST). GLAST is expressed by Muller cells, which keeps the level of glutamate below neurotoxic levels. (24,25)

High glucose concentration causes reduced glutamate uptake and decreased GLAST expression and there is increased generation of ROS (26). High levels of glutamate cause excitotoxicity and disruption of retinal neurons, causing neural apoptosis. High glutamate leads to activation of NMDA receptor, which stimulates nitric oxide synthase, which results in increased production of nitric oxide. Excess nitric oxide combines with superoxide free radicals to form peroxynitrate. This compound attacks pericytes and endothelial cells as a powerfull cytotoxin.

Excess glutamate also cause NMDA receptor activation. This results in increased intracellular calcium, that activates endogenous phospholipase enzyme. This enzyme degrades membrane phospholipids and produces oxygen derived free radicals and prostaglandin. All these cause damage to the lipid rich retinal membrane. Lipid peroxides are generated which are toxic to retinal cells (27).

Lactate also influences the increased expression of VEGF from retinal neurons in a concentration dependent way (28). Excess unutilized glucose stimulates non enzymatic glycation of proteins along with reactive AGE formation. These are postulated to cause long time diabetic microvascular complications (29). Circulating AGEs causes activation of reduced NADPH oxidase in pericytes, EC, mesangial cells and macrophages. This causes increased ROS production and cellular oxidative stress (30). This causes pericyte dysfunction and loss, as well as basement membrane thickening, blood retinal barrier dysfunction, microaneurysm formation and capillary drop out, in early stage of DR (31).

V. CONCLUSION

The increased generation of VEGF, lactate and increased intracellular calcium, extracellular glutamate, nitrate, MDA, AGEs and ROS, all cause death of pericytes of the vascular endothelium of retinal capillaries. There is vascular apoptosis, ischaemia and breakdown of inner retinal barrier. All these biochemical alterations play their significant roles in the causation of diabetic retinopathy. The assessment of these biomarkers may provide early information so as to predict the onset of microangiopathy in type 2 DM and thus, appropriate preventive action may be taken.
Figure 1: Spectrofluorometer

Figure 2: Flow Cytometry

Figure 3: Randox – LC 2389, UK

Figure 4: Nonproliferative diabetic retinopathy (NPDR)
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