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Analyze the status of 5'methylcytosine DNA methylation level in the CpG Island of promoter region of BDNF gene for the pathogenesis of Type II Diabetic Retinopathy

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[Accredited with "A" grade by NAAC]

ABSTRACT: Type 2 Diabetic retinopathy (T2DR) is a sight threatening, microvascular complication of diabetes that affects the retina. It is the most common complication of diabetes and a leading cause of blindness amongst working aged adults in the developed world. It is broadly recognized as a neurovascular disease. Patients suffering from T2DR are one third of human population suffering from diabetes mellitus. Retina is a neuronal tissue of the eye produces neurotrophic factors for its maintenance. Brain-derived neurotrophic factor (BDNF) is one of the neurotrophic factors that support differentiation and maturation. BDNF helps retinal cell survival in a concentration dependent Manner. BDNF may protect retina from hyperglycemia and provides pathogenesis of T2DR. DNA methyltransferases (Dnmts) catalyses methylation of cytosine to 5-methyl cytosine (5mC). Dnmts a family with five different members—Dnmt1, Dnmt2, Dnmt3a, Dnmt3b and Dnmt3L; out of which only Dnmt1 Dnmt3a and Dnmt3b are catalytically active. It has been observed that level of BDNF dysregulates during the pathogenesis in T2DR patients. Present review concluded that the level of 5' methyl cytosine DNA methylation on BDNF in a retinal cell affects the microvasculature of human retina that can named as neovascularization or any retinal microvasculopathy. The analysis of 5' methyl cytosine DNA methylation on BDNF between the patient and a healthy person can be important in the establishment of cause of T2DR and may provide hints about their unique role in the pathogenesis of disease.

KEYWORDS: Type 2 Diabetic Retinopathy, Epigenetic, DNA Methylation, Brain derived neurotrophic factor, DNA methyltransferase.

I. INTRODUCTION

Type II Diabetic retinopathy is the leading cause of acquired blindness in working adults. The microvasculature of the retina is damaged, the blood vessels swell and leak fluid and if not prevented, new vessels start to grow, and ultimately lead to the detachment of the retina. It is a duration-dependent disease that develops in stages; the incidence of retinopathy is rarely detected in the first few years of diabetes, but the incidence increases to 50% by 10 years, and to 90% by 25 years of diabetes (1). This slow progressing disease is mainly characterized by damage of the microvasculature of the retina. Although hyperglycemia is the major initiator of diabetic retinopathy, hypertension and dyslipidemia are also considered significant risk factors in its development and progression (2). Epigenetic mechanisms regulate gene functions without changing the nucleotide sequence of DNA. These regulations are reversible, and the most commonly studied epigenetic mechanisms are DNA methylation and histone modification

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(3).DNA methylation, addition of a methyl group on position 5 of cytosine residues of the cluster of CpG dinucleotides (CpG Island).Cytosine 5 methylation generates 5-methylcytosine (5mC), and the reaction is catalyzed by a family of enzymes— DNA (cytosine-5) methyltransferases (Dnmts) (2). DNA methylation is a heritable epigenetic mark involving the covalent transfer of a methyl group to the C-5 position of the cytosine ring of DNA by DNA methyltransferases (DNMTs) (4).In case of T2DR under the conditions of hyperglycemia retinal endothelial cell were used as a model to study apoptosis in diabetic retinas. Brain derived neurotrophic factor (BDNF) mainly abundant in blood serum supplies over retina through microvascular system and effective in protecting retina from hyperglycemia.(5). From this study we may analyse the status of 5'methylcytosine DNA methylation level in the CpG Island of promoter region of BDNF gene.

II. TYPE II DIABETIC RETINOPATHY

T2DR, a disease of the retina, is the leading cause of acquired blindness in working adults.The microvasculature of the retina is damaged, the blood vessels swell and if not prevented, new vessels start to grow, and ultimately lead to the detachment of the retina. It is a duration-dependent disease that develops in stages; the incidence of retinopathy is rarely detected in the first few years of diabetes, but the incidence increases to 50% by 10 years, and to 90% by 25 years of diabetes.The prevalence of diabetic retinopathy is increasing due to prolonged survival of diabetic patients(1).It has been the leading cause of blindness amongst 16- to 64-year-olds(6). Due to high circulating glucose, the tiny blood vessels that nourish the retina are damaged. If not controlled, this non-proliferative stage could progress to proliferative stage where the new vessels begin to grow, ultimately resulting in retinal detachment and blindness (7).

In the pathogenesis of diabetic retinopathy, retinal mitochondria become dysfunctional, initiating the apoptotic machinery, and capillary cell apoptosis precedes the development of retinal histopathology associated with diabetic retinopathy(8) (fig 1). Almost all individuals with insulin-dependent diabetes mellitus (IDDM) will develop some form of a macrovascular or microvascular complication within 15 years of their diagnosis, with a higher occurrence in type I than in type II diabetes (9). Macrovascular complications of diabetes include coronary heart disease, peripheral artery disease, stroke, and many other disorders. Microvascular complications of diabetes can also lead to diabetic neuropathy, diabetic nephropathy, or diabetic retinopathy(10). Diabetic retinopathy is a serious complication of diabetes and is the leading cause of new-onset blindness in working-age adults (11). Although diabetes is characterized by hyperglycemia, DR will often continue to progress even after tight glycemic control is achieved(12).Type 2 Diabetic Retinopathy classified into two clinical forms they are: non proliferative diabetic retinopathy, proliferative diabetic retinopathy. Non proliferative diabetic retinopathy subdivided into 3 different stages they are mild, moderate and severe. These stages are marked by progressive alterations to the retinal microvasculature that contribute to the pathology of DR. Newly formed vessels in the diabetic retina are fragile and leaky to fluid and protein, which leads to macular edema (retinal thickening), blood pooling in the tissue, and substantial visual impairment. Several factors are known to increase the risk of developing DR. These include the duration of diabetes, poor glycemic control, high blood pressure, high cholesterol, pregnancy, elevated glycated hemoglobin-A1c (HbA1c) and tobacco and tobacco use(13).Oxidative stress is regarded as one of the leading mechanisms in its development, and increase in oxidative stress in diabetic milieu is associated with a number of other interlinking metabolic abnormalities including the accumulation of advanced glycation end products, activation of protein kinase C, polyol and hexosamine pathways.

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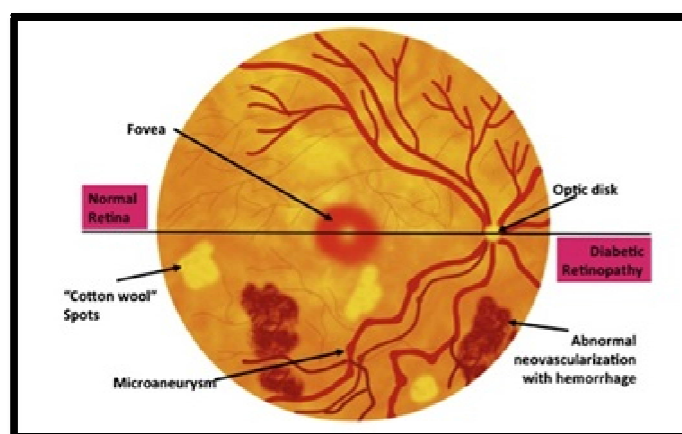


Fig 1: Diabetic retinopathy is marked by microvascular dysfunction including abnormal neovascularization, hemorrhages, microaneurysms & cotton wool spots that will eventually, if not treated lead to vision loss.

III.EPIGENETICS MECHANISM

Epigenetics is presently described as the study of changes in gene expression that occur not by changing the DNA sequence, but by modifying DNA methylation and remodeling chromatin. The major epigenetic mechanisms are DNA methylation, histone modifications and modulation of gene transcription and translation by non-coding RNAs, including miRNAs. These epigenetic changes are potentially reversible and modulated by the environment, diet or pharmacological intervention (14). The epigenetic of T2DR is the interaction between gene activation and epidemiology, where gene activation can be in the form of DNA methylation, histone modification or RNA activation. This could be affected by different epidemiological factors, namely age, obesity, nutrition, physical activity and intrauterine environment (15).

IV.DNA METHYLATION AND ITS BASIC FUNCTION

DNA methylation is a major epigenetic mechanism which involves direct chemical modification to the DNA. Historically, DNA methylation was discovered in mammals as early as DNA was identified as the genetic material (16). In 1948, Rollin Hotchkiss first discovered modified cytosine using paper chromatography in a preparation of calf thymus. Hotchkiss (1948) assumed that this element was 5-methylcytosine (5mC) was separated from cytosine that was similar to the thymine (also known as methyl uracil) which was separated from uracil, and this modified cytosine existed naturally in DNA (17). Although many researchers proposed that DNA methylation might regulate gene expression, it was not until the 1980s that several studies demonstrated that DNA methylation was involved in gene regulation and cell differentiation (18). DNA methylation is catalyzed by DNA methyltransferases (Dnmts) that is a family of enzyme that transfer a methyl group to the fifth carbon of a cytosine residue to form 5mC.

DNA methylation, in general, is defined as a biochemical process whereby a methyl group is added to DNA bases. It usually occurs on the fifth position of cytosine (C) to form 5-methyl cytosine, known as 5mC in the context of CpG dinucleotides—regions of DNA where a cytosine nucleotide is followed by a guanine nucleotide in the linear sequence of bases along its 5' to 3' direction in both eukaryotes and prokaryotes. The effect of 5mC is traditionally considered to interfere with transcriptional initiation, thereby silencing gene expression (19).

In case of T2DR, DNMTs enzyme activity is increased in the retina and its capillary cells and the expression of DNMT1 (protein and gene), but not of Dnmt3a or 3b, is also remarkably elevated (20). Hence, it proved that DNMT1 continues to be over expressed even after termination of hyperglycemia, signifying its role in the metabolic memory phenomenon. Consistent with results, differential DNA methylation patterns are observed in the blood samples of patients with proliferative diabetic retinopathy (21).

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V. BRAIN DERIVED NEUROTROPHIC FACTOR

Brain-derived neurotrophic factor (BDNF) is one of the neurotrophic factors that support differentiation (22), maturation (23), and survival of neurons in the nervous system (24) and shows a neuroprotective effect under adverse conditions, such as glutamatergic stimulation, cerebral ischemia, hypoglycemia, and neurotoxicity. BDNF stimulates and controls growth of new neurons from neural stem cells (neurogenesis) (25). BDNF control the development, plasticity and survival of cholinergic, dopaminergic, and serotonergic neurons. It also controls glutamatergic neurotransmitter which release and promotes the development of GABAergic neurons. Human BDNF consists of complex gene structure of 11 exons (I–V, Vh, VI–VIII, VIIIh, IX), 9 of which (exon I–VII, IX) contain functional promoters (26). The use of translation start sites in exons I, VII, and VIII of BDNF can lead to the pre-pro BDNF proteins with longer N-termini. And different splice sites are situated in exons II, V, VI, and IX. BDNF gene most abundant in retina any Dysregulation in the level of BDNF may leads T2DR.

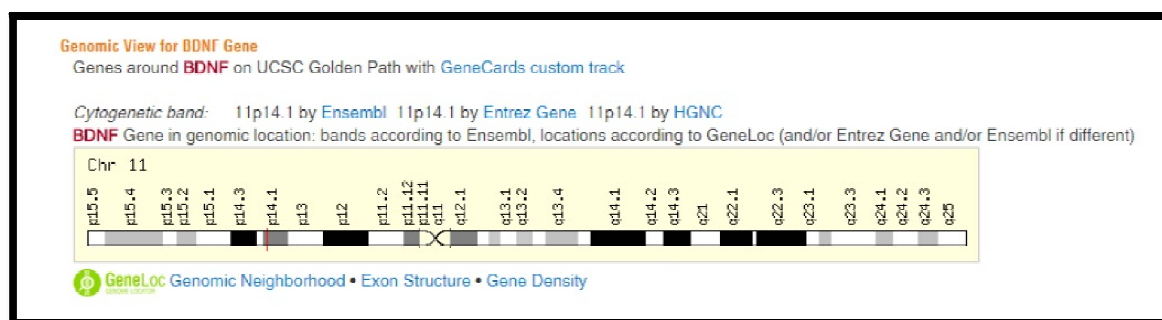


Fig 2: Structure of BDNF gene

In the animal model of T2DR, few studies showed that reduced level of BDNF in the T2DR may damage neurons, thereby leading to neurodegeneration(27). The patients with T2DR were found to have a drastic reduction in the level of BDNF in retina (28). BDNF levels are lower in individuals with T2DM compared to non-diabetic individuals, both in plasma and serum, and in different populations (29). It has been observed that difference in serum BDNF levels, DNA methylation patterns and genetic variants, across different glucose metabolic state advice that BDNF may be involved in the pathophysiological(30).

VI.CONCLUSION

Epigenetic mechanisms are hypothesized to play an important role in the case of Type 2 Diabetic Retinopathy because they provide a mechanism for continued altered gene expression. It has been reported that hyperglycemia can induce site-specific DNA methylation. Hyperglycemia, a condition where glucose level increases leads to epigenetic modification like DNA methylation which then alters BDNF gene expression and due to that BDNF protein level changes which may lead to T2DR.

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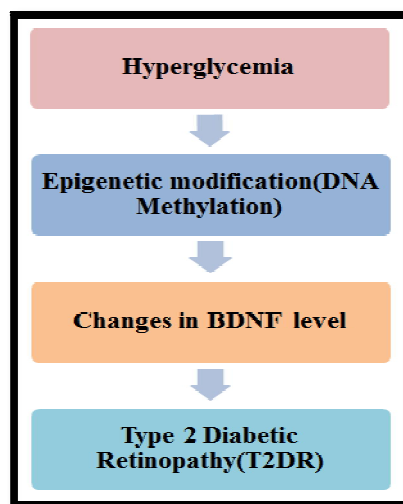


Fig-3: Flow chart of T2DR

The available literature reviewed thoroughly concluded that level of DNA methylation in BDNF in retinal cells may affect the microvasculature of human retina that can be named as neovascularization or any retinal microvasculopathy. The Expression level of DNA methylation of BDNF gene between the patient and a healthy person can be important in the establishment of cause of Type 2 Diabetic Retinopathy and hints about their unique role in the pathogenesis of disease.

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