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ETIOLOGICAL, BIOCHEMICAL, AND PATHOPHYSIOLOGICAL CHANGES DURING GESTATIONAL DIABETES LEADING TO NEURAL TUBE DEFECTS IN FOETUSES – A REVIEW

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ABSTRACT

Background Among the several risk factors, maternal diabetes mellitus during pregnancy is considered as an important risk factor for the incidences of neural tube defects (NTDs). The prevalence of hyperglycaemia in pregnant women was 16.9% affecting ~21 million live births globally (2013- report). Further the scope of NTDs in these women was reported to be ten times higher than in non-diabetic pregnant women. Hence the objective of this article is to elucidate a comprehensive review on the biochemical, cellular and molecular mechanisms involved in causation of NTDs in hyperglycaemic environment during pregnancy. **Key Findings** It was observed that maternal hyperglycaemia is a marker for metabolic abnormalities which results in enhanced polyol pathway; inositol depletion; altered arachidonic acid metabolism and formation of advanced glycation end products. These underlying metabolic abnormalities in gestational diabetic women affects various signaling molecules, transcription factors that regulate neurulation processes, cell proliferation and differentiation of neural stem cells in the developing fetus leading to the development of NTDs. Details of these mechanisms and how they are interlinked with gestational diabetes is described in this article. **Conclusion** This review summarizes the possible biochemical mechanisms involved in aetiology of hyperglycaemia induced NTDs. Having reviewed the scenario of the probable causes of NTDs, it is concluded that there is an imperative need for identifying novel diagnostic markers for early detection of NTDs thus offering hope to gestational diabetic women to deliver a child free from NTDs. Some of the probable biomarkers are being highlighted through this investigation.

KEYWORDS: Neural tube defects; Gestational Diabetes Mellitus; Advanced Glycation End Products; Neurulation; Arachidonic acid; Diagnostic markers.

1. INTRODUCTION

Congenital anomalies or birth defects are a cluster of disorders of prenatal origin caused by single gene defects, chromosomal defects, multifactorial inheritance, environmental teratogens and micronutrient deficiencies. Maternal viral infections such as syphilis, rubella, maternal illnesses like diabetes mellitus, exposure to drugs like valproic acid and conditions such as iodine, folic acid deficiency are other factors that cause birth defects. Birth defects came into our knowledge after the famous international thalidomide tragedy was reported, an anti-nausea, anti-convulsant drug widely prescribed during pregnancy, caused thousands of infants to be affected by phocomelia between 1958 and 1962.[1] Among the various defects some are caused by the hyperglycaemia, the two most common malformations are congenital heart defects and neural tube defects (NTDs).

In this article we propose to understand the birth defects –if any caused by Gestational diabetes mellitus (GDM). Pregnant women who develop this are generally due to glucose intolerance with onset or first recognition during pregnancy of variable severity. Among the many causes it is reported due to altered physiology during pregnancy which causes insulin resistance when the pancreas fails to compensate the increasing demands for insulin. It occurs as a part of adaptive mechanism with an intention to provide abundant nutrients to the developing foetus. Insulin resistance occurs due to release of various hormones from the placenta such as progesterone, prolactin, lactogen and cortisol possessing anti-insulin effects.[2] Pre-existing diabetes, obesity, adiposity serve as additional factors contributing for the development of insulin resistance during pregnancy.[3] This leads to an increased risk of congenital anomalies such as NTDs in the early developmental stages. During Embryogenesis a

portion of embryonic ectoderm folds to form a neural plate which elevates and undergoes fusion to form the neural tube. Formation of neural tube is known as neurulation, the neural tube eventually forms the central nervous system in the embryo. This entire process is regulated and coordinated by various transcription factors. Deregulation of such events which occurs during cell proliferation, migration, differentiation and apoptosis in neural progenitor cells under maternal hyperglycaemic environment leads to neural tube defects. NTDs are congenital abnormalities of the brain and vertebral column occurring due to improper or non-timely closure of the neural tube during embryogenesis; having multi-factorial, yet unknown etiologies resulting in anencephaly or exencephaly and spina bifida. Neural tube closure occurs within 3-4 weeks of pregnancy after conception in humans.[4] Previous studies in mice reported that mutations in more than 30 genes are responsible for failure of neural tube closure.[5] Anencephaly is a disorder wherein major portion of brain, skull and scalp are absent. It occurs when rostral (head) end of neural tube fails to close between 23rd and 26th day following conception. Exencephaly is a condition where the brain is located outside the skull. Spina bifida occurs when vertebrae overlying spinal cord are malformed and they remain unfused and open. The mechanisms by which these birth defects arise in the presence of high maternal glucose level are being unraveled. While numerous distinct causes of NTDs have been identified, the etiopathogenesis of gestational diabetes linked NTDs remains frustratingly unexplained. A further obstacle is that most of the women with diabetes do not seek preconceptional care and most of them may have unplanned pregnancies.[6] Women past the crucial time of neural tube closure (3-4 weeks of pregnancy) need pre pregnancy glycaemic control to reduce the risk of NTDs. The scope of the article is to elucidate a comprehensive review on the cellular and molecular changes involved in NTDs causation and pathogenesis under hyperglycemic environment.

2. Maternal Hyperglycaemia and Birth Defects

Birth Defects the leading cause of infant mortality contribute more than 3 million deaths across the world. The prevalence of hyperglycaemia in pregnancy was 16.9%, affecting ~21 million live births in 2013 globally. The estimated prevalence for hyperglycaemia associated birth defects ranges from 2-6 per 1000 births depending on geographical and ethnic background. Adverse glycaemic control in pregnancy has 3 fold increased risk for major cardiovascular, gastrointestinal and renal defects in infants[7] and contribute to approximately 40% of the neonatal mortality. Pre-gestational diabetes increases the risk of NTDs by two to tenfold.[8] The second most common debilitating birth defect, neural tube defects (NTDs) affects approximately 3, 00,000 births globally each year.[9] Birth defects in diabetic mothers encompass of both structural and functional abnormalities. Structural abnormalities include absence of femur, genital abnormalities, ventricular septal defect, nervous system abnormalities and pulmonary artery stenosis. Functional abnormalities include macrosomia, hyperbilirubinemia and cardiomyopathy.

3. Pathogenesis of Gestational diabetes linked Neural Tube defects

Innumerable teratological pathways have been suggested, often from retrospective clinical studies experience, and characterized subsequently in various experimental systems. Major teratogenic processes so far identified in embryonic tissues include alterations of metabolic pathways such as enhanced polyol pathway leading to accumulation of sorbitol, inositol depletion, alterations in arachidonic acid/prostaglandins levels, as well as in protein kinase C (PKC) isoforms. The formation of advanced glycation end products in embryo, the maternal and foetal genotypes involved in neurulation, neuronal cell proliferation, neuronal apoptosis are proposed to influence the teratological events in diabetic pregnancy.

3.1 Polyol pathway

Polyol pathway converts glucose into sorbitol catalysed by aldose reductase enzyme. Under normal conditions aldose reductase enzyme does not metabolise glucose. Improper glycaemic control in tissues results in enhanced polyol pathway ascribed to accumulation of sorbitol creating an intracellular hypertonic state and compensatory efflux of myo-inositol resulting in reversible damage at first and later progresses to irreversible structural derangement leading to growth retardation and dysmorphogenesis in the foetus. Exposure to hyperglycaemic environment enhanced sorbitol formation in embryos and supplementation with aldose reductase inhibitors in pregnant diabetic animals lowered sorbitol accumulation, restored inositol levels and diminished the occurrence of congenital anomalies.[10]

3.2 Inositol depletion

Embryos cultured on high glucose media yielded an embryonic deficiency of inositol due to impaired uptake[11] concomitantly leading to embryonic dysmorphogenesis. Myo-inositol causes partial hydrolysis of phospholipids and forms phosphoinositides and diacylglycerol (DAG). DAG is involved in maintaining membrane integrity by controlling Na^+/K^+-ATPase activity. Embryonic deficiency of inositol causes alterations in membrane potential and increases in membrane permeability to proteins, other macromolecules leading to deleterious cell lesions. This is evident from the studies that supplementation of inositol to embryos cultured on high glucose[12] or addition of inositol to diabetic pregnant rodents through diet[13] reduced the inositol deficiency and the rate of embryonic dysmorphogenesis. Addition of the scyllo-inositol (inositol uptake inhibitor) to the culture medium of rodent embryos causes analogous changes to the embryos, i.e. both inositol deficiency and embryonic dysmorphogenesis.[14] These findings suggest that

inositol deficiency is one of the key components contributing to diabetic teratogenesis.

3.3 Altered protein kinase C activity (PKC)

Evidence from the experimental work on embryonic mice exposed to a hyperglycaemic environment showed two strong impacts on PKC activation: one is inhibitory via decreased PKC activation by reducing inositol availability leading to decreased phoshoinositides and the other pathway is stimulatory via augmented glycolytic flux leading to increase in production of DAG which activates several PKC isoforms.[15] Maternal diabetic environment alters intracellular signaling of PKC family.[16] PKC comprises of twelve isoforms in which PKCα, PKCβ2, and PKCδ are found to be associated with NTDs in diabetic embryopathy.[17] Increased DAG level in embryonic neural tube activates PKCβ2 and it initiates an apoptotic pathway via caspase 3, 8, Bid and cytochrome C thereby causing excessive apoptosis leading to neural tube defects.[18] Activated PKCs also elicit the generation of reactive oxygen species[19] leading to a vicious cycle in which increased oxidative stress results in NTDs.

3.4 Arachidonic acid deficiency

For the normal development of palate, neural tube, mandible, genitals and heart, phosphatidyl inositol turnover coupled to the arachidonic acid cascade, leading to prostaglandin synthesis is required. Altered metabolism of arachidonic acid and prostaglandins in experimental diabetic pregnancy led to congenital anomalies.[20] Intraperitoneal injections of arachidonic acid to pregnant diabetic rats diminished the rate of neural tube damage, thereby indicating disturbance in the arachidonic acid cascade as a consequence of a diabetic environment.[21] Diabetic environment down regulated embryonic cyclooxygenase-2(cox-2) gene expression that lowered ProstaglandinE_2 (PGE_2) levels and resulted in embryonic dysmorphogenesis. Studies also reported that decreased concentrations of PGE_2 in diabetic environment obstruct neural tube closure.[22]

3.5 Advanced Glycation End Products (AGEs)

AGEs play a crucial role in diabetic embryopathy. The formation of AGEs accelerates under the hyperglycemia. The incidence of congenital abnormalities occurs when chronic poor glycemic control predates conception.[23] α-oxoaldehyde precursors of AGEs such as glyoxal, methylglyoxal, and 3-deoxyglucosone (3-DG) are increased in many tissues in the diabetic state.[24] 3-DG modifies guanylyl nucleotides[25] and this modification on DNA, histones cause errors in replication and transcription thereby promoting mutations favouring apoptotic cell death[26] and leading to congenital anomalies.[27] Receptor for advanced glycation end products (RAGE) is a multi-ligand member of the immunoglobulin super family. RAGE expression is increased in diabetes and inflammation and it promotes cellular dysfunction by binding to AGE ligand.[28] Studies on rodent models demonstrate that binding of S100/ calgranulins to RAGE in embryonic cells of the developing nervous system increase the expression of RAGE thereby stimulating neurite growth and neuron survival.[29] Increased binding of AGE to RAGE as a consequence of diabetic environment resulted in foetal malformations. This is evident from studies on RAGE knockout mice diminished the rates of fetal malformations and resorptions, despite similar levels of hyperglycemia in pregnant diabetic mice.[30] Therefore it seems that AGEs/RAGE interaction plays an important role in the normal neural cell differentiation.

3.6 Oxidative Stress

Maternal hyperglycaemia delivers high glucose to embryos. The increased intracellular glucose metabolism through hypoxia[31] or hexosamine biosynthetic pathway[32] leads to oxidative stress in embryos resulting in mitochondrial dysfunction, lipid peroxidation and DNA damage. Increased oxidative stress results in excessive neuroepithelial apoptosis in embryos due to reduced expression of pax-3 and increased expression of p53 lead to NTDs.[33] Earlier studies reported that the frequency of embryonic malformations in diabetic pregnancy markedly reduced by dietary supplements rich in antioxidants such as vitamin C[34] suggesting that oxidative stress is involved in the etiology of embryonic dysmorphogenesis.

3.7 Glucose Transporters

Due to down regulation in expression of glucose transporters Glut1, Glut2 and Glut3 under diabetic environment glucose uptake in embryos is reduced and it triggers the onset of early apoptosis resulting in embryonic dysmorphogenesis by loss of vital progenitor cells in blastocysts mass.[35] Glut1 expression is altered in the neural tube of rodent embryos during early organogenesis.[36] Hyperglycaemic environment initially up regulates the expression of Glut1in neural stem cells indicating an increase in glucose transport to embryo resulting in sorbitol accumulation and oxidative stress. Oxidative stress in latter phase down regulates the Glut1 transporter in neural stem cells exposed to hyperglycaemic environment reduces glucose transport to embryos thereby impairing the expression of genes associated with cell proliferation of neural stem cells ultimately leading to NTDs.

It is evident from the above data that diabetes induced dysmorphogenesis in embryo is associated with metabolic abnormalities such as enhanced polyol pathway, depletion of inositol in embryos, decreased phospholipid turn over and protein kinase-c activation. Alteration in arachidonic acid metabolism, formation of advanced glycation end products and oxidative stress finally leads to interruption in signal transduction ultimately resulting in apoptotic death.

3.8 Abnormal Neurulation in embryos of Diabetic Pregnancy

Maternal hyperglycaemia not only causes aberrant glucose metabolism and insulin secretion but also affects the foetus nervous system. Persistent hyperglycemia during critical periods of development elicits malformations in the foetal brain leading to dysmorphogenesis. Signaling molecules, genes and transcription factors that regulate forebrain patterning were altered under hyperglycaemic environment during embryogenesis.[37] Shh (Sonic hedgehog) a protein secreted from two signaling centers the notochord and floor plate regulates the proliferation of neural precursors.[38] It induces the expression of NKx 2.1and NKx 2.2 (homeo box transcription factor). NKx 2.1 and Brain Factor- 1(BF-1) vital for the development of telencephalon and retina were increased in embryos of diabetic mice[39] leading to forebrain malformations. Pax-3 (Paired box a transcription factor) required for neural tube closure in the area of mid brain and hind brain. It acts by inhibiting p53 dependent apoptosis.[40] In embryos of diabetic mice Pax-3 was down regulated[41] thereby resulting in increased apoptosis, alterations in cell migration, faulty pyrimidine synthesis attributing to development of NTDs.

Transthyretin, a protein produced by choroid plexus epithelial cells is essential for the transport of retinol and thyroxine from peripheral circulation to brain and it regulates neuronal differentiation in the developing brain.[42,43] Decreased availability of transthyretin under maternal hyperglycaemic environment attributes to impairment in the development of essential parts of brain such as hypothalamus and medulla oblongata leading to intellectual impairment in the off-spring of diabetic mothers.[44]

3.9 Apoptosis and cell proliferation genes alteration

Cell death is crucial for normal development and for maintaining tissue homeostasis. Among the various types of cell death, which include autophagic cell death and necrosis, apoptosis is observed widely in physiological tissue turnover and in multicellular organisms during development.[45] Embryos from diabetic mice exhibit increased apoptosis on the surface of neural folds during organogenesis.[41] Deregulation of apoptosis or suppression of cell proliferation in embryos directly results in congenital anomalies. Embryos cultured with high glucose levels show cell death in the neural tube.[46] Earlier studies reported that increased rates of apoptosis occur in preimplantation embryos of diabetic rodents, which may be one of the contributing mechanisms that induce fetal miscarriages.[47] In mammalian blastocysts, diabetic environment modulated p-53 expression,[48] increases the expression of the proapoptotic protein Bax,[35] and decreased the expression of Bcl-2 mRNA.[49] This findings represent that Bax-mediating apoptosis has an important role in diabetic-induced apoptosis at the blastocyst stage. Maternal glucose intolerance during embryogenesis also impairs cell proliferation and induces apoptosis of neural stem cells derived from telencephalon of mouse embryos.[50] Previous studies on diabetic mouse demonstrated that hyperglycemia induced death of neural crest cells resulted in impaired development of neural crest derived cranial ganglia VII-X.[51] Over all it appears that maternal hyperglycemic environment alters the expression of developmental control genes which might induce pro-apoptotic genes leading to excessive apoptosis in the developing neural tube.

Cyclins and cyclin dependent kinases (CDK) act as regulators of cell cycle. Cyclins drive cells from the G1-S-G2-M phase by binding to cyclin dependent kinases. $P57^{kip2}$ is a cyclin dependent kinase inhibitor its interaction with cyclin-E-CDK2, D2-CDK4 function as a negative regulator of cell proliferation.[52] After neural tube closure $P57^{kip2}$gets expressed and inhibits transition from G1 to S phase. Down regulation of $P57^{kip2}$ in diabetic environment promotes cell transition from G1 to S phase during neural tube closure period interfering with differentiation of neural progenitor cells resulting in NTDs.[53] CDK5 plays a vital role in the developing nervous system as evident from the studies on CDK5 knockout mice depicted significant embryonic defects.[54] CDK5 is involved in neuronal survival, cell migration, synaptic functions, axonal growth and even it regulates the neuronal apoptosis by regulating Bcl2 expression.[55] Down regulation of CDK5in diabetic environment may promote the development of NTDs by causing increased apoptosis. The effect of $P57^{kip2}$ and CDK5 on nervous system is evident from studies performed on retinoic acid induced NTDs on rats.[56] These changes suggest that maternal diabetic environment alters the cell cycle progression in the neural tube by regulating the expression of genes involved in apoptosis and cell proliferation which concomitantly result in neural tube malformations in embryos of diabetic mice. This could be due to the fact that development of various regions of the brain is controlled by a distinct set of signalling molecules and transcription factors, which may exhibit differential response to the glucotoxicity. Over all, the disturbance in normal cycle of cell death and proliferation of neural precursor cells and their progenies in the developing neural tube by hyperglycemia may contribute to the defective patterning and the subsequent complex neural tube anomalies in embryos of diabetic pregnancies.

3.10 Insulin and insulin-like growth factor 1 (IGF-1)

Insulin and insulin-like growth factor 1 (IGF-1) is involved in regulation of brain development. IGF-1 and insulin vary in their functions though they possess similar amino acids and trigger convergent signaling pathways.[57] Generally, insulin control glucose metabolism, whereas IGF-1 exhibits the features of trophic factors and it provoke the differentiation of choroid plexus epithelial cells during development.[58] IGF signalling exhibits pleiotropic actions on neural stem cells, post mitotic neurons, astrocytes, oligodendrocytes

and restricted lineage neural precursor cells. By interacting with type 1 IGF receptor (IGF1R) it promotes proliferation, maturation, survival and growth of neural cells. IGF-1 promotes cell survival by its anti-apoptotic action.[59] It accelerates neurogenesis by reducing G1 phase length and increase the re-entry of cell cycle.[60] It promotes dendrites branching, elongation,[61] synaptogenesis[62] and axon myelination.[63] Maternal hyperglycaemic environment resulted in aberrant Insulin/IGF-1 signals in embryonic brain that led to retardation of dendritic development in foetus.[64] IGF-1 or IGF-1 receptor (IGF-1R) knockout mice depicted the severe growth-retardation, brain retardation and incomplete myelination compared with their wild-type counterparts.[65,66] Maternal hyperglycemia induced by direct injection of glucose or β-cell disruption from streptozotocin treatment in rodents resulted in exencephaly in the offspring. Explanted rodent embryos grown in high glucose media also exhibit a failure of neural tube closure[67] and this is due to alteration in mRNA expression of IGFs during maternal diabetes.[68]

3.11 Transforming Growth Factor- β

Neurogenesis involves neural stem cells proliferation, differentiation, maturation, integration into neural circuits and cell cycle arrest. Transforming Growth Factor- β (TGF-β) play a vital role in the regulation of neurogenesis, homeostasis of motor neurons, schwann cell proliferation in the peripheral nervous system.[69] Three forms of TGF-β exists TGF-β1, TGF-β2 and TGF-β3 and are produced from glial and neuronal cells.[70] Administration of anti- TGF-β type II receptor antibody blocked the activity of TGF-β and resulted in impaired neurogenesis.[71] DAG-protein kinase C pathway, the polyol pathway and the glucosamine pathway play an important role for the increased production of TGF-β1 during diabetes and this may contribute for intrauterine growth retardation[72] and excessive apoptosis concomitantly leading to NTDs. Bone morphogenetic protein 4 a member of the TGF- β superfamily, is involved in patterning of the dorsal neural tube is decreased in the telencephalon in embryos of diabetic pregnancy leading to fore brain malformations.[73]

Taken together maternal hyperglycaemia leads to oxidative stress which further alters the expression of genes necessary for cell proliferation, apoptosis and growth factors during early embryogenesis and neurulation ultimately resulting in NTDs.

3.12 Folic acid

Folic acid requirement is high during pregnancy as it plays a vital role in organogenesis. Folic acid is involved in proliferation[74] and differentiation of neuronal progenitor cells. It also promotes neurogenesis by stimulating Erk 1 or 2 (Extracellular Signal Regulated Kinases) phosphorylation and notch signalling. Maternal hyperglycaemia leads to functional folic acid deficiency in embryo during organogenesis[75] resulting in excessive apoptosis[41] thereby affecting CNS development. Folic acid supplementation regulates apoptosis by altering Bax and Bcl-2.[76] Neural progenitor cells undergo differentiation and produces neurons and glial cells. The formed neurons and glial cells fate is decided by basic helix-loop helix (bHLH) transcription factors.[50,77] bHLH factors such as Neurogs and Mash initate neurogenesis. NeuroDs mediate terminal differentiation[77] and useful for development and survival of cerebellar and hippocampal neurons.[78] Supplementation with folic acid during pregnancy upregulated bHLH transcription factors[78] Doublecortin (DCX) is a protein expressed by neuronal precursor cells serve as a marker for neurogenesis. Folic acid even regulates the neuronal migration by increasing the DCX expression.[79] Folic acid deficiency during pregnancy results in NTDs by vague mechanisms. The possible mechanisms include decrease in availability of methyl groups, accumulation of homocysteine and by impairing the synthesis of thymidine.

4. Diagnostic tests

The diagnosis of Gestational Diabetes Mellitus (GDM) is based on the Oral Glucose Tolerance Test and glycosylated haemoglobin levels. The values of Glucose tolerance test is depicted in table: 1. The incidence of major congenital anomalies is observed when glycosylated haemoglobin levels are in the range of 8.6 to10.0 per cent or higher.[80] Pre-natal diagnosis of congenital anomalies is done by assessing serum alpha fetoprotein (AFP) level, ultra sound monitoring of foetal growth and amniotic fluid volume every 4 weeks between 28 and 36 weeks. AFP is formed from the yolk sac and the liver during foetal development. The normal range of AFP in maternal serum is 0.5 to 2.0 or 2.5 MoM (Multiple of the Median). High levels of AFP in maternal serum indicate that the developing baby has a neural tube defect such as spina bifida or anencephaly and also suggest oesophageal defects or a failure of baby's abdomen to close. However, the limitation of this test is elevated AFP levels observed due to inaccurate dating of the pregnancy. Low levels of AFP, abnormal levels of human chorionic gonadotropin (hCG) and estriol may specify that the developing baby has Trisomy 21(Down syndrome), Trisomy 18 (Edwards Syndrome) or other chromosome abnormality.[81] Currently transabdominal ultrasonography combined with endovaginal ultra sonographic imaging is useful to detect congenital anomalies. Endovaginal ultra sonographic imaging is useful to identify fetal cardiac defects.[82]

Table 1: Diagnostic criteria (Glucose Tolerance test) for GDM.[85]

Glucose load (Grams)	Plasma Glucose Values				Criteria	Steps and increased values to consider pathology
	Fasting Mmol/l	1hour Mmol/l	2 hour Mmol/l	3 hour Mmol/l		
75	7.0	-	7.8	-	WHO	1
75	5.1	10.0	8.5	-	IADPSG	1
100	5.3	10.0	8.6	7.8	Coustan & Carpenter	2
100	5.8	10.6	9.2	8.1	NDDG	2
100	5.0	9.2	8.1	6.9	O'Sullivan	2

WHO — World Health Organization; NDDG —The National Diabetes Data Group; IADPSG — The International Association of Diabetes and Pregnancy Study Groups.

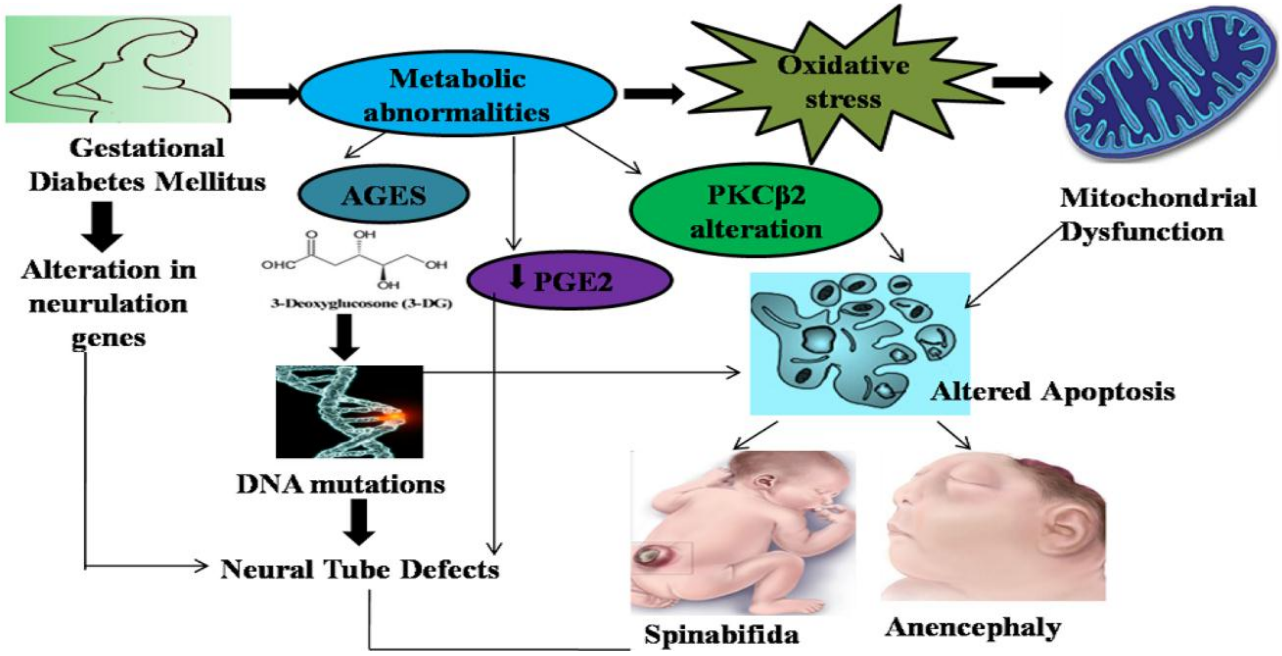

Figure 1: pathogenesis of gestational diabetes mellitus linked neural tube defects.

5. Therapeutic Interventions

Therapeutic approach is early detection of GDM to reduce the maternal hyperglycaemia as it leads to teratogenic condition as pre-existing tissue damage cannot be reversed even after achieving normoglycaemic levels. Insulin therapy has been the practice of clinicians to initiate monitoring when there is an evidence of fetal macrosomia. Metformin is another logical option recommended for treating maternal hyperglycaemia as it improves insulin sensitivity, and is not associated with adverse effects such as hypoglycaemia or maternal weight gain. However, it does cross the placental barrier and may alter foetal physiology. Previous work also described that herbal extracts such as *Abroma augusta* and *Ocimum sanctum* reduced blood glucose levels.[83,84] As oxidative stress is implicated in the pathogenesis of diabetic embryopathy earlier intervention studies with dietary supplements rich in antioxidants such as N-acetyl cysteine, zinc, folic acid, vitamin C, and curcumin lowered the frequency of gestational diabetes induced neural tube defects. Moreover diabetic environment induces a state of functional folic acid deficiency in the embryo during early organogenesis. This deficiency is partly a transport and concentration deficiency.[75] Supplementation with folic acid is the corner stone therapy prescribed before conception and during pregnancy to reduce the risk for neural tube defects.

6. CONCLUSION

Complications in early developmental stages have been described by several authors however in this review we have highlighted all the possible biochemical, morphological, cellular and molecular changes involved in NTD causation under maternal hyperglycaemic environment. Metabolic disturbances, oxidative stress, impaired gene expression and consequent apoptosis during embryogenesis, or disturbed organogenesis under hyperglycaemic milieu may be general mechanisms that explain diabetic embryopathy. Since embryogenesis and neurulation is a multifaceted process involving various transcription factors and signaling factors we have tried to unveil how diabetic environment may lead to NTDs. Pre-natal diagnosis along with knowledge of genetic and

molecular mechanisms may offer hope to gestational diabetic women to deliver a child free from NTDs.

AUTHOR CONTRIBUTIONS

All the authors mentioned contributed substantially to the writing and revising of the manuscript.

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CONFLICTS OF INTEREST

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