Frontiers in research on maternal diabetes-induced neural tube defects: Past, present and future

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Abstract
Diabetes mellitus rightly regarded as a silent-epidemic is continually on the rise and estimated to have a global prevalence of 6.4 % as of 2010. Diabetes during pregnancy is a well known risk factor for congenital anomalies in various organ systems that contribute to neonatal mortality, including cardiovascular, gastrointestinal, genitourinary and neurological systems, among which the neural tube defects are frequently reported. Over the last two to three decades, several groups around the world have focussed on identifying the molecular cues and cellular changes resulting in altered gene expression and the morphological defects and in diabetic pregnancy. In recent years, the focus has gradually shifted to looking at pre-programmed changes and activation of epigenetic mechanisms that cause altered gene expression. While several theories such as oxidative stress, hypoxia, and apoptosis triggered due to hyperglycemic conditions have been proposed and proven for being the cause for these defects, the exact mechanism or the link between how high glucose can alter gene expression/transcriptome and activate epigenetic mechanisms is largely unknown. Although preconceptual control of diabetes, (i.e., managing glucose levels during pregnancy), and in utero therapies has been proposed as an effective solution for managing diabetes during pregnancy, the impact that a fluctuating glycemic index can have on foetal development has not been evaluated in detail. A tight glycemic control started before pregnancy has shown to reduce the incidence of congenital abnormalities in diabetic mothers. On the other hand, a tight glycemic control after organogenesis and embryogenesis have begun may prove insufficient to prevent or reverse the onset of congenital defects. The importance of determining the extent to which glycemic levels in diabetic mothers should be regulated is critical as foetal hypoglycemia has also been shown to be teratogenic. Finally, the major question remaining is if this whole issue is negligible and not worthy of investigation as the efficient management of diabetes during pregnancy is well in place in many countries.

Key words: Maternal diabetes; Congenital anomalies; Neural tube defects; Hyperglycemia; Hypoxia; Oxidative stress; Neural stem cells; Epigenetics; Epigenome

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EPIDEMIOLOGY
The global incidence of diabetes mellitus is constantly on the rise and is estimated to be 6.4% as of 2010[10]. According to the World Health Organization statistics...
2012 report, diabetes is prevalent in one in ten adults. The incidence of diabetes in pregnancy however, varies with the geographical and ethnic background of women. About 3%-5% of pregnancies are reported to be complicated by diabetes mellitus which is the leading cause for mortality and morbidity[2,3].

Diabetes during pregnancy is of serious concern as it causes spontaneous abortions, still birth, foetal macrosomia, and congenital malformations[4-7]. Our understanding and management of diabetes over the years has reduced the risk for spontaneous abortion and still birth in maternal diabetics. However, even in recent years diabetes during pregnancy has been considered to have a teratogenic effect causing foetal anomalies[7-11]. Although all of the foetal tissues are susceptible to glucose induced anomalies, the developing heart and brain have shown maximum defects[12] and these defects are the most frequently reported birth defects in humans and mouse models of diabetic pregnancy[5,13-15], indicating that these tissues are more sensitive to glucose toxicity.

Indeed, initial studies have shown that the type of malformation varied with the maternal glucose concentration[6,16] and achieving a particular glucose threshold may be beneficial in reducing or eliminating the risk for a particular malformation. While a tight glycemic control started before pregnancy has shown to reduce the risk for congenital anomalies[6,10,17,18], the same after organogenesis (first 8 wk of pregnancy) has taken place may prove insufficient to reverse the damages already caused. While majority of the pregnancies are diagnosed only after 7-8 wk of conception, in a mother with undiagnosed and pre-existing diabetes, organogenesis in the fetus is definitely impaired and is irreversible. Neural tube defects (NTDs), the second most common birth defect caused by maternal diabetes has been long studied and characterised. In this review, we summarize some of the major findings of the past and present and propose future directions for research to alleviate NTDs.

MORPHOLOGICAL AND MOLECULAR CHARACTERISATION OF NTDs

Maternal diabetes has been shown to cause severe patterning defects in the brain of developing embryos. We have reported maternal diabetes-induced malformations in several parts of the brain including the forebrain (telencephalon), spinal neural tube, choroid plexus (CP) and ventricles in mouse embryos[15,28]. The impaired development of CP by maternal diabetes results in decreased production of cerebro-spinal fluid which is proposed to cause defective patterning and shaping of the brain during development in diabetic pregnancy[21-24]. In addition, the neural tissue with defective patterning showed altered expression of several signalling molecules and transcription factors such as Shh, Nkx2.1, bafilomycin-1, transforming growth factor-β and Pax3 which are critical in forebrain patterning and neural tube closure[25-28].

Recently, high throughput gene expression profiling using cDNA microarray revealed the altered expression of several genes in the cranial neural tubes of embryos from diabetic pregnancy[20,28-30]. Higher numbers of genes involved in metabolism and cellular process were found to be altered by maternal diabetes[29]. Microarray analysis on whole embryos from diabetic pregnancy has also revealed the altered expression of several genes involved in critical developmental pathways that could contribute to maternal diabetes-induced birth defects including genes known to cause NTDs[29]. These descriptive reports suggest that brain development is impaired due to the altered expression of developmental control genes caused by maternal diabetes-induced glucotoxicity.

CHARACTERISATION OF METABOLIC PATHWAYS IN NTDs

In recent years, the research on maternal diabetes-induced congenital malformations in fetus has moved on from morphological characterisation to identifying the etiology of birth defects. It has been widely shown that maternal diabetes induces hypoxia, oxidative stress and other metabolic disturbances in the embryo[30] and these changes alter several signalling pathways and molecules which have been proposed to be the major causative factors for the diabetes-induced malformations leading to embryopathy. For example, the oxidative stress caused by hyperglycemia has been shown to disrupt the expression of genes such as Pax3 that is involved in neural tube formation which explains in part that hyperglycemia-induced oxidative stress alters gene expression leading to NTD[32].

Hyperglycemia-induced birth defects are attributed to the excessive production of reactive oxygen species (ROS) which has been shown to cause oxidative stress and subsequently increase the risk for fetal malformations[33,34]. Administration of antioxidants such as vitamin E and overexpression of ROS scavenging enzymes such as superoxide dismutase have been shown to prevent or reduce the risk for diabetic malformations in several animal studies although the exact mechanism is unknown[19-46].

Development and patterning of normal brain depend on proliferation and differentiation of neural stem cells (NSCs) which are self renewing multipotent cells giving rise to neuronal and non-neuronal cells (glial cells such as astrocytes and oligodendrocytes) in the central nervous system. We have shown that NSCs are extremely sensitive to glucotoxicity, which alters the expression of genes involved in proliferation and lineage specification of NSCs[41]. Apoptosis in the neuroepithelium is the hallmark of maternal diabetes-induced NTDs and the balance between cell proliferation and cell death that is altered by hyperglycemia may contribute to the malformations seen in the developing neural tube. We have also shown that high glucose induces ROS production and intracellular oxidative stress in NSCs by increasing glucose reduction via the polyol pathway. These changes appeared to be mediated by aldose reductase (AR), the rate
limiting enzyme in the polyol pathway, since its expression was found to be increased in NSCs exposed to Hg in vitro and inhibition of AR using fidarestat, reversed the changes induced by Hg \[^{12}\]. Overall, these studies indicate that the development of strategy to prevent oxidative stress during fetal development may alleviate the risk of congenital anomalies in embryos.

**UNRAVELLING EPIGENETIC MECHANISMS CONTRIBUTING TO NTDS**

It has been reported that maternal nutrition and metabolic disturbances during fetal development can alter epigenetic mechanisms such as histone modifications and DNA methylation in fetus, and such epigenetic changes may have long lasting effects on the offspring postnatally \[^{43-46}\]. Study on pregnant mice fed with diets low in choline/methionine have resulted in decreased methylation in genes that control brain development \[^{47,48}\], and altered memory and long-term potentiation \[^{49,50}\] indicating that the developing embryo is influenced by maternal diet. In addition, diabetic rodents supplemented with folate (a methyl donor) prevented NTDs in the embryos \[^{51,52}\] that define the role of maternal nutrition on fetal outcome.

It has been widely shown that diabetic complications are associated with epigenetic modifications. In recent years, several reports have shown that the onset of diabetes in adults is caused by DNA methylation at specific gene promoters or chromosomal regions \[^{53-56}\] signifying epigenetic basis for onset of diabetes in adults. High glucose has also shown to cause persistent alterations in gene expression through histone modifications (by acetylation or methylation of lysine residues) during transient exposure of human aortic endothelial cells \[^{57}\] and chronic exposure of human monocyte cell line \[^{58}\] to high glucose.

Further, excess glucose has been shown to increase histone acetylation in mammalian cells \[^{59}\] while excess dietary methyl donors increase DNA/histone methylation in offspring \[^{60}\] providing evidence for the relationship between maternal hyperglycemia (or diet) and fetal epigenome. Recently, glucose responsive microRNAs such as miR-26a, miR107 and miR-16 that show increased expression in high glucose conditions have been identified \[^{61}\] suggesting that epigenetic mechanisms could be activated by hyperglycemia. Further, epigenetic factors have been shown to regulate gene expression of developmental control genes and fate specification of NSCs \[^{62,63}\]. It is possible that high glucose modifies epigenetic mechanisms which subsequently alter expression of genes involved in cell fate specification of NSCs thereby resulting in NTDs. Further investigation of epigenetics will be useful to understand the relationship between maternal diet and the fetal epigenome. It will be intriguing to know how high glucose/hyperglycemia activates epigenetic mechanisms that alter gene expression in the fetus resulting in birth defects.

**CONCLUSION**

Diabetes during pregnancy is a well known teratogen that causes congenital anomalies. Over the last 2-3 decades, the focus on this field of research has shifted from morphological and molecular characterisation of the etiology of maternal diabetes-induced malformation to understanding the mechanisms behind how maternal diabetes alters fetal development. While epigenetic mechanisms have been proposed to modify the expression of critical genes involved in development, the exact mechanism behind this still remains largely unidentified. It will be interesting to elucidate how epigenetic mechanisms such as DNA methylation, chromatin/histone modifications and microRNAs are activated in embryonic tissue by maternal diabetes. Identifying specific histone/DNA modifying enzymes involved in response to glucose prove as significant therapeutic targets, since epigenetic changes are reversible. Modulating fetal epigenetic programming in such metabolic syndromes may prove valuable to improve fetal outcomes or prevent onset of diabetes in offspring of diabetic mothers.

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