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Original Research

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Birth defects in newborns: Spina bifida index at Rio Grande Do Norte State in Brazil

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ABSTRACT

Objective: Among birth defects (BD) or congenital anomalies, spina bifida (SB) is the most common congenital malformation of the central nervous system that is associated with significant lifelong morbidity. We aimed to obtain statistics index of SB in newborns at Rio Grande do Norte (RN) state, Brazil, as well as to identify perinatal risk and to update therapeutic options to minimize the mortality in newborn with SB congenital defects. **Materials and Methods:** Retrospective study of SB index in newborn in Pediatric Hospitals at RN State, Brazil, over the 6 years from 2008 to 2013 and literature review about SB global status was made at PubMed database, reference lists of selected publications and important research groups in the field of SB. **Conclusion:** Northeast region is the one that has the major incidence of SB in Brazil country, but RN state has a number lower than others states from its region. It was made an update about therapeutic options to minimize the morbidity and mortality in newborn with SB congenital defects.

KEY WORDS: Folic acid, neural tube defects, spina bifida

INTRODUCTION

Birth defects (BD) or congenital anomalies include all structural and functional alterations in embryonic or fetal development resulting from genetic, environmental or unknown causes, which result in physical and/or mental impairment; neural tube defects (NTDs) are common complex congenital malformations resulting from failure of the neural tube closure during embryogenesis. With a worldwide prevalence of approximately 1/1000 births, depending on geographic region and ethnical grouping, making them the second most common congenital malformations, following congenital heart defects [1], and Spina bifida (SB) is the most common congenital malformation of the central nervous system (CNS) that is associated with significant lifelong morbidity [2-4]. The causes of NTD are multifactorial. The evidence for genetic predisposition as a determinant for NTD is: A preponderance of NTD in females; prevalence differences related to racial and ethnic background; an increased prevalence in siblings. Environmental risk factors for NTD are the use of anti-epileptic drugs and maternal conditions such as diabetes, hyperthermia, obesity and certain professions e.g. agriculture or cleaning [5,6].

It is established that folic acid (FA) supplementation during pregnancy decreases (50-70%) the risk of NTDs, which has led to national public health policies regarding FA [7,8]. The name FA was given by Mitchell, Snell and Williams [9] to a growth-factor for the micro-organism *Streptococcus faecalis* R (originally but erroneously regarded as a strain of *Streptococcus lactis*); it was found in spinach and concentrated to a high degree of purity. Many animal studies have provided sufficient information to establish the metabolic and/or genomic mechanism(s) underlying human FA responsiveness in NTDs. However, several lines of evidence suggest that not only folates, but also choline, B12 and methylation deficiencies in metabolisms are involved in NTDs. Decreased B12 vitamin and increased total choline or homocysteine in maternal blood have been shown to be associated with increased NTDs risk. During pregnancy, fetal growth causes an increase in the total number of rapidly dividing cells, which leads to increased requirements for folate. Inadequate folate intake leads to a decrease in serum folate concentration, resulting in a decrease in erythrocyte folate concentration, a rise in homocysteine concentration, and megaloblastic changes in the bone marrow and other tissues with rapidly dividing cells [10-12]. According Yan *et al*., [13] intestinal folate absorption was enhanced by erythropoietin (EPO) treatment *in vitro*, and its findings provided direct evidence to establish the correlation between EPO and folate homeostasis. EPO up regulated the transport of FA across caco-2 cells, which was associated with the modulation on the expression of folate transporters.

Several polymorphisms of genes involved in these pathways have also been implicated in risk of development of NTDs. The supplementation with B12 vitamin, betaine or other methylation donors in addition to FA periconceptional

supplementation will further reduce NTD risk. The protective effect of FA on the occurrence and recurrence of NTDs has been clearly demonstrated 20-25 years ago. FA has a direct effect on the neurulation stage embryo, as treatment of genetically predisposed mouse embryos *in vitro* can normalize neural tube closure [14-26].

Low-density lipoprotein receptor-related protein 2 (LRP2) is a multifunctional cell-surface receptor expressed in the embryonic neuroepithelium. Loss of LRP2 in the developing murine CNS causes an impaired closure of the rostral neural tube at embryonic stage. LRP2 is essential for cellular FA uptake in the developing neural tube, a crucial step for proper neural tube closure [27].

FA enters on carbon (carbohydrate) metabolism, which has two main outputs: Production of pyrimidines and purines for DNA replication during cell proliferation and donation of methyl groups to macromolecules including DNA, proteins and lipids. Cell multiplication plays a key role in neural tube closure, encouraging the hypothesis that enhanced cell proliferation may be a key effect of FA. On the other hand, methylation of genomic DNA and histones is increasingly being implicated in the (epigenetic) regulation of gene expression 48, and could underlie the action of FA in preventing NTDs [28,29]. Besides, some evidence links folate deficiency with colorectal cancer incidence [30-32].

In addition, stimulation of adenosine monophosphate-activated protein kinase inhibits the expression of *Pax*3, a gene that is essential for neural tube closure, and induces NTDs [33,34]. The receptor kinase domain provides sites for association with adaptor proteins. Moreover, evidence from recent reports suggests that ErbB2/4 receptors, through their C-terminal amino acids, can form specific associations with scaffolding proteins. The existence of such assemblies expands the range of signaling cascades available to the Neuregulins (NRGs). NRGs are a large group of structurally related signaling proteins that are likely to have important roles in the development, maintenance and repair of the nervous system and other selected tissue. Their receptors are the ErbB protein tyrosine kinases, which are important in cell signaling [35].

This study is designed to obtain SB statistics index in newborns at RN state, Brazil, as well as to identify perinatal risk and to update therapeutic options to minimize the morbidity and mortality in newborn with SB congenital defect.

MATERIALS AND METHODS

This work is a retrospective study and all data are obtained of SB cases in newborn, which are diagnosed at Pediatric Hospitals at Rio Grande do Norte (RN) State (27 number of cases) in Northeast region (2.536 number of cases), Brazil country (5.488 number of cases), over the 6 years (from 2008 to 2013). Source: SUS database (www.saude.gov.br) and PubMed database, reference lists of selected publications and important research groups in the field.

Statistical Analysis

Data are expressed as means ± standard error of mean and comparisons among groups analyzed using one-way analysis of variance, followed by the Student-Newman-Keuls test for multiple comparisons.

RESULTS

The incidence of SB is higher at Northeast region in Brazil country, but RN state has a number lower than others states from its region [Figure 1].

The effect of four polymorphic genes of folate-dependent methionine biosynthesis has been investigated in mothers affected by an NTD pregnancy and matched controls. The influence of the various genotypes on total red cell 5-methyl-H(4) folate,5,10-methenyl-H(4) folate, and 5-formyl-H(4)folate is reported, as is the effect on homocysteine and radioassay folate in both serum and red cells. In NTD-C677T 5,10-methylene tetrahydrofolate reductase in particular, indexes of folate depletion such as high-performance liquid chromatography folate level, oligo-gamma-glutamyl chain length, homocysteine, and radioassay folate values all seem to deteriorate with increased mutant allele carriage. Furthermore, a trend that is less universal indicates that NTD mothers have higher 5,10-methenyl-H(4) folate and 5-methyl-H(4) folate levels and lower 5-formyl-H (4) folate and H (4) PteGlu (1) levels than do controls. One of the most consistent, and possibly specific, differences between participant groups is a statistically significant elevation of 5,10-methenyl-H(4) folate in NTD mothers, affects three genotypes. In addition, prenatal ultrasound screening for external ear abnormalities and evaluate the feasibility of examining the fetal external ear with ultrasonography, as well as magnetic resonance imaging are techniques to better analyze and understand the problems of patients with SB [5,36-44].

Unfortunately, at RN state, only image diagnosis by regional medical imaging and ultrasound is made to SB diagnostic.

DISCUSSION

SB is the most common congenital malformation of the CNS that is associated with significant lifelong morbidity.

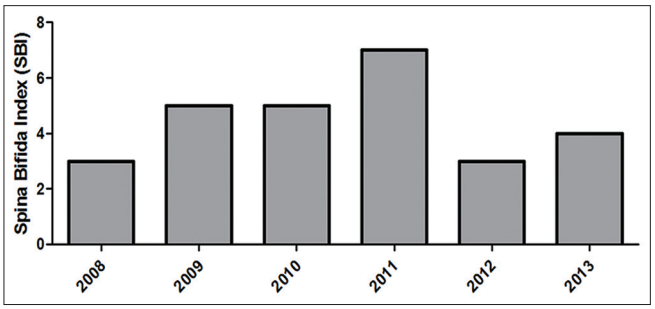

Figure 1: Spina bifida index at Rio Grande do Norte state, Brazil (from 2008 to 2013). Source: SUS database (www.saude.gov.br)

The northeast region in Brazil country is the one that has the major incidence of SB, but RN state has a number lower than others states from its region. Appropriate therapies such as FA supplementation could reduce the risk of NTDs during pregnancy. Mandatory fortification of flour with FA has proved to be one of the most successful public health interventions in reducing the prevalence of NTD-affected pregnancies. Most developing countries have few, if any, common sources of FA, unlike many developed countries, which have FA available from ready-to-eat cereals and supplements. Expanding the number of developed and developing countries with FA flour fortification have tremendous potential to safely eliminate most FA - preventable NTDs [45-53].

The close liaison between pediatric surgeons and colleagues in the fetomaternal medicine enabled fetal defects to be diagnosed with ultrasonography and the clinical course and pathophysiology of many of these disorders to be observed. The antenatal detection of a fetal structural abnormality often entails referral to a pediatric surgeon, who can counsel parents and discuss the likely outcomes for the fetus. For some structural abnormalities, such as SB, fetal surgery repair the SB defect in uterus and stops spinal cord destruction as well as restores normal neurological function at birth [3,54,55]. In addition, the potential use of neural stem cells and genetic therapies for the fetus is emerging as a way to improve the treatment of SB and hydrocephalus, and so prevent the disease or diminish/repair the associated brain damage [56,57]. Several congenital diseases are particularly attractive candidates for intervention using gene therapy since the underlying molecular bases for most of the monogenic disorders are well-understood. Transplantation of *ex vivo* genetically modified stem cells has also shown promise. The great potential of induced pluripotent cells is that it allows the possibility of deriving pluripotent stem cells from any human patient. Generation of patient-derived stem cells serves as a great source for developing cell replacement therapies and also for creating human cellular model systems of specific diseases or disorders [58-60].

The number of mouse mutants and strains with NTDs exceeds 240, including 205 representing specific genes, 30 for unidentified genes, and 9 multifactorial strains. These mutants identify genes needed for embryonic neural tube closure. The genetic basis of mammalian normal neural tube closure has the potential to become one of the more well-understood developmental morphogenetic processes, and a detailed and sophisticated developmental genetic study of this bounty of mutants is essential. These genes appear to be conserved across species [61-63].

The initial step in neural tube development (neurulation) is a characteristic thickening of the ectoderm from the level of the primitive node of Hensen caudally to the prochordal plate rostrally at the beginning of the 3rd week of embryonic life. This slipper-shaped structure is called the neural plate. Studies in *Xenopus* embryos led to the belief that the ectoderm is preprogrammed toward a neural fate, and endogenous bone morphogenetic proteins (BMPs) inhibit this default system. BMPs are multi-functional growth factors that belong to the transforming growth factor beta superfamily. The roles of BMPs in embryonic development and cellular functions in postnatal and adult animals have been extensively studied in recent years. Signal transduction studies have revealed that Smad1, 5 and 8 are the immediate downstream molecules of BMP receptors and play a central role in BMP signal transduction. Studies from transgenic and knockout mice and from animals and humans with naturally occurring mutations in BMPs and related genes have shown that BMP signaling plays critical roles in heart, neural and cartilage development. BMPs also play an important role in postnatal bone formation. BMP activities are regulated at different molecular levels. Preclinical and clinical studies have shown that BMP-2 can be utilized in various therapeutic interventions such as bone defects, non-union fractures, spinal fusion, osteoporosis and root canal surgery. Until date, around 20 BMP family members have been identified and characterized. BMPs signal through serine/threonine kinase receptors, composed of Type I and II subtypes. Three Type I receptors have been shown to bind BMP ligands, Type IA and IB BMP receptors (BMPR-IA or anaplastic lymphoma kinase [ALK]-3 and BMPR-IB or ALK-6) and activin receptor type IA (ActR-IA or ALK-2). However, neural induction in higher vertebrates appears to be a more complex process, in which inhibition of BMP-4 involves an intricate interplay of FGF (fibroblast growth factors), Noggin, Chordin, Wnt-3, β-catenin and possibly calcium transients [59,64-66].

Differential rates of cell proliferation, cell movement and changes in cell shape in the neural plate result in the formation of the neural groove in the median plane and neural folds on either side. By complex processes of cell-shaping (apicobasal elongation, apical narrowing and basal expansion), cell movement and cell adhesion, aided by the forces provided by the underlying mesenchyme and overlying surface ectoderm, the neural folds elevate themselves, converge along the dorsal midline and fuse with each other to form the neural tube [63,67-69]. Fusion also involves the surface ectoderm at the edges of the neural folds dorsal to the neural tube. Neural crest-derived ectomesenchyme in the cranial region and somitic mesenchyme in the trunk region spread around the neural tube under the surface ectoderm and form the primordia of the meninges, axial skeleton and muscles attached to the axial skeleton. For example, BMP signaling has been implicated in most aspects of craniofacial skeletogenesis, including PA1 development [70-74]. The development of the craniofacial muscles requires reciprocal interactions with surrounding craniofacial tissues that originate from cranial neural crest cells (CNCCs). However, the molecular mechanism involved in the tissue-tissue interactions between CNCCs and muscle progenitors during craniofacial muscle development is largely unknown [75,76].

Kang *et al*. [77] first described a thermo labile variant of the methyltetrahydrofolate reductase (MTHFR) which interferes with folate and remethylation pathways. This variant is due to the 677C >T polymorphisms (Ala222Val, rs1801133) and was first associated with NTD risk by Van der Put *et al*. [78]. It leads to mild to highly increased plasma total homocysteine concentrations depending on the folate status.

Numerous studies have looked at the relationship between NTD risk and genotypes of mothers with children with NTD or children affected with NTD. Several meta-analyses have been performed, and all have found a significantly increased risk of NTDs associated with MTHFR 677C > T polymorphisms in either maternal or paternal genotypes. The strength of this association was probably influenced by the ethnic origin of the populations studied and the riboflavin as well as the folate status. Following the identification of the MTHFR 677C > T variant, many potential polymorphisms in genes involved in folate, remethylation, B12 and choline pathways have been explored in NTDs [79,80]. There is a consensus that periconceptional folate is essential for proper neurodevelopment, and research suggests that a sufficient amount of supplemental FA in the 1st month of pregnancy may reduce the child's susceptibility for autism spectrum disorder (ASD) [81,82]. ASD is characterized by communication impairments, social abnormalities, and stereotypic behaviors, and several medical comorbidities are observed in autistic individuals. The etiological basis of ASD is unclear, and evidence suggests it involves both genetic and environmental factors; and it is characterized by certain physiological abnormalities, including oxidative stress, mitochondrial dysfunction and immune dysregulation/inflammation [83-88], as well as acquired by immunization [89-93].

In addition, the advances in gene therapy hold significant promise for the treatment of neurological disorders such as Alzheimer's disease, epilepsy and neurological manifestations in lysosomal storage disease [94,95], hematological disease [96,97], ophthalmic conditions such as heritable diseases of the retina [98-100], endocrinology [101], rheumatic diseases [106-108], diseases of the gastrointestinal tract [102-112], therapy of cancer [113-117], and bone and joint disorders [118].

Another important real find is neural stem cells hold considerable promise for the treatment of the injured or degenerating nervous system, their current human sources are embryonic stem cells and fetal derived neural tissue. Spinal cord injury (SCI) has remained a challenging area for scientists and clinicians due to the adverse and complex nature of its pathobiology. Until date, clinical therapies for debilitating SCI are largely ineffective. However, emerging research evidence suggests that the repair of SCI can be promoted by stem cell-based therapies in regenerative medicine. Over the past decade, therapeutic potential of different types of stem cells for the treatment of SCI has been investigated in preclinical models. These studies have revealed multiple beneficial roles by which stem cells can improve the outcomes of SCI. The major focus is the future role of stem cell transplantation and similar rehabilitative restorative approaches designed to optimize spontaneous regeneration by mobilizing endogenous stem cells and facilitating other cellular mechanisms of regeneration, such as axonal growth and myelination [119-125].

The hair follicle bulge area is an abundant, easily accessible source of actively growing, pluripotent adult stem cells. Nestin, a protein marker for neural stem cells, is also expressed in follicle stem cells as well as their immediate differentiated progeny. The nestin-expressing hair follicle stem cells differentiate into blood vessels, neurons, glial cells, keratinocytes, smooth muscle cells and melanocytes *in vitro*. Human hair follicle stem cells as well as mouse hair follicle stem cells promote nerve repair and can be applied to test the hypothesis that human hair follicle stem cells can provide a readily available source of neurologically therapeutic stem cells. The optimal source of stem cells for regenerative medicine is a major question. The use of hair follicle stem cells for nerve regeneration overcomes critical problems of embryonic stem cells or induced pluripotent stem cells in that the hair follicle stem cells are multipotent, readily accessible, non-oncogenic, and are not associated with ethical issues [126-131]. In addition, current studies indicate nestin-expressing fungiform papilla cells and the nestin-expressing hair follicle stem cells have common features of cell morphology and ability to differentiate into multiple cell types, suggesting their remarkable similarity [132-134].

Finally, the understanding of music's role and function in therapy and medicine is undergoing a rapid transformation, based on neuroscientific research showing the reciprocal relationship between studying the neurobiological foundations of music in the brain and how musical behavior through learning and experience changes brain and behavior function. Music therapy (MT) treatment improves depressive symptoms, sleep quality and others mental and behavioral disorders, and it could help both baby and mother's mental health to have a safe pregnancy [135,136].

One of the primary goals of cognitive neuroscience is to understand the interaction between genes, development and specific experience. A particularly fascinating example of this interaction is a sensitive period - a time during development when experience has a differential effect on behavior and the brain. Behavioral and brain imaging studies in musicians have provided suggestive evidence for a possible sensitive period for musical training; showing that musicians who began training early show better task performance and greater changes in auditory and motor regions of the brain. Several studies comparing adult musicians and non-musicians have provided compelling evidence for functional and anatomical differences in the brain systems engaged by musical training. It is not known, however, whether those differences result from long-term musical training or from pre-existing traits favoring musicality. In an attempt to begin addressing this question, we have launched a longitudinal investigation of the effects of childhood music training on cognitive, social and neural development [137-141] as well as to player rehabilitation after neurologic injury [142].

This study regarded to revise about SB global status, and specifically at RN state. Northeast region is the one that has the major incidence of SB in Brazil country, but RN state has a number lower than others states from its region; Moreover, appropriates therapies such as FA supplementation, which reduces the risk of NTDs, and MT are safe to use during pregnancy.

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