NEURAL TUBE CLOSURE DEFECTS: EMBRYOLOGICAL, CLINICAL AND PREVENTIVE ASPECTS

LOS DEFECTOS DEL CIERRE DEL TUBO NEURAL: ASPECTOS EMBRIOLOGICOS, CLINICOS Y PREVENTIVOS

DEFEITOS DE FECHAMENTO DO TUBO NEURAL: ASPECTOS EMBRIOLÓGICOS, CLÍNICOS E PREVENTIVOS

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ABSTRACT

Thanks to the progressive advancement of prenatal genetic diagnosis techniques, both from the clinical, biochemical and genetic point of view, the detection of certain diseases of the embryo and fetus has improved, particularly central nervous system anomalies. The introduction of ultrasonography has allowed early diagnosis of these anomalies, minimizing the need for invasive procedures that could compromise fetal viability (Bianchi et al., 2019). Advances in ultrasound have been spectacular, the result of the efforts of numerous researchers who have developed equipment with greater resolution capacity, making it possible to evaluate the product of gestation from its embryonic stage. These new procedures have significantly improved diagnostic accuracy and the identification of complications in a large number of diseases of the central nervous system, in particular neural tube closure defects (NTDs). Therefore, in this research the pathologies related to NTDs are reviewed, with a special focus on the biomolecular aspects of embryonic development, epidemiological factors and the usefulness of screening by alpha-fetoprotein (AFP) in maternal serum and amniotic fluid, as well as high-resolution ultrasound as a complementary diagnostic method. In addition, preventive strategies to reduce the incidence and recurrence of these anomalies are addressed. This research represents a significant contribution to the understanding of the pathologies that can be diagnosed by biochemical and ultrasound screening, facilitating their follow-up and management by professionals in obstetrics, perinatology and genetics.

Keywords: Prenatal genetic diagnosis. Neural tube closure defects (NTCD). High resolution ultrasound. Fetal central nervous system.

RESUMEN

Gracias al progresivo avance de las técnicas de diagnóstico genético prenatal, tanto desde el punto de vista clínico, bioquímico y genético, se ha mejorado la detección de ciertas enfermedades del embrión y del feto, particularmente de las anomalías del sistema nervioso central. La introducción de la ultrasonografía ha permitido la realización de diagnósticos precoces de estas anomalías, minimizando la necesidad de procedimientos invasivos que podrían comprometer la viabilidad fetal (Bianchi et al., 2019). Los avances en la ecografía han sido espectaculares, resultado del esfuerzo de numerosos investigadores que han desarrollado equipos con mayor capacidad resolutiva, permitiendo evaluar el producto de la gestación desde su etapa embrionária. Estos nuevos procedimientos han mejorado significativamente la precisión diagnóstica y la identificación de complicaciones en un gran número de enfermedades del sistema nervioso central, en particular los defectos del cierre del tubo neural (DCTN). Por ello, en esta investigación se revisan las patologías relacionadas con los DCTN, con un enfoque especial en los aspectos biomoleculares del desarrollo embrionario, los factores epidemiológicos y la utilidad del cribado mediante alfa-fetoproteína (AFP) en suero materno y líquido amniótico, así como la ecografía de alta resolución como método diagnóstico complementario. Además, se abordan estrategias preventivas para reducir la incidencia y recurrencia de estas anomalías Esta investigación representa un aporte significativo para la comprensión de las patologías que pueden ser diagnosticadas mediante cribado bioquímico y ecográfico, facilitando su seguimiento y manejo por parte de profesionales en obstetricia, perinatología y genética.

Palabras clave: Diagnóstico genético prenatal. Defectos del cierre del tubo neural (DCTN). Ecografía de alta resolución. Sistema nervioso central fetal.

RESUMO

Graças ao avanço progressivo das técnicas de diagnóstico genético pré-natal, do ponto de vista clínico, bioquímico e genético, a detecção de determinadas doenças do embrião e do feto melhorou, principalmente as anomalias do sistema nervoso central. A introdução da ultrassonografia permitiu o diagnóstico precoce dessas anomalias, minimizando a necessidade de procedimentos invasivos que poderiam comprometer a viabilidade fetal (Bianchi et al., 2019). Os avanços na ultrassonografia foram espetaculares, como resultado dos esforços de vários pesquisadores que desenvolveram equipamentos com maior capacidade de resolução, permitindo a avaliação do produto da gestação desde o estágio embrionário. Esses novos procedimentos melhoraram significativamente a precisão do diagnóstico e a identificação de complicações em um grande número de doenças do sistema nervoso central, em particular os defeitos de fechamento do tubo neural (DTNs). Portanto, nesta pesquisa, revisamos as patologias relacionadas aos DTNs, com foco especial nos aspectos biomoleculares do desenvolvimento embrionário, nos fatores epidemiológicos e na utilidade da triagem de alfa-fetoproteína (AFP) no soro materno e no líquido amniótico, bem como no ultrassom de alta resolução como método de diagnóstico complementar. Além disso, são abordadas estratégias preventivas para reduzir a incidência e a recorrência dessas anomalias. Esta pesquisa representa uma contribuição significativa para a compreensão das patologias que podem ser diagnosticadas por meio de triagem bioquímica e ultrassonográfica, facilitando o acompanhamento e o gerenciamento por profissionais de obstetrícia, perinatologia e genética.

Palavras-chave: Diagnóstico genético pré-natal. Defeitos de fechamento do tubo neural (DTN). Ultrassom de alta resolução. Sistema nervoso central do feto.

INTRODUCTION

Neural tube closure defects (NTCDs) are congenital malformations resulting from a failure in neural sulcus development around day 28 of embryonic development (1). Approximately half of the cases of DCTN correspond to anencephaly, while the rest include encephalocele and spina bifida (2). Despite advances in research, the specific causes of DCTN remain unfully defined, due to the interaction between genetic and environmental factors that are not yet fully understood (3).

Prenatal screening for DCTN is performed by determining alpha-fetoprotein (AFP) levels in maternal serum and amniotic fluid (LA), as well as by high-resolution ultrasound, which allows the diagnosis to be accurately defined (4). Scientific evidence has shown that folic acid (FA) administration significantly reduces the incidence of these birth defects, which has led to the implementation of strategies to increase folate intake in the female population (5)).

The approach recommended by public health agencies is for all women of reproductive age who have not had a previous pregnancy with DCTN to consume 0.4 mg of FA daily at least four weeks before conception and until the end of the first trimester (6). In women with a history of pregnancies affected by DCTN, the recommended dose is 4 mg daily to prevent recurrence (7).

APPROACH TO THE PROBLEMATIC SITUATION

Neural tube closure defects are pathologies of the central nervous system that have a prevalence of 1 to 2 per 1000 live births, constituting a significant public health problem (8). Among the main risk factors is the deficiency of folic acid and folate in the diet, which has motivated the implementation of food fortification programs to reduce the occurrence and recurrence of these malformations (9).

In Ecuador, congenital defects, and in particular DCTN, represent a major problem due to their high morbidity and mortality rates, as well as the serious disabling sequelae they generate. In addition to the impact on patients' quality of life, these malformations impose a considerable emotional and economic burden on families and society (10).

In 2006, 100 cases of TNCD were reported in the province of Guayas, with an incidence rate of 7.8 per 10,000 live births (11). However, since 2008 there has been a slight decrease in the number of cases, attributable to significant underreporting and deficiencies in reporting, making it difficult to know the true scale of the problem.

To address this problem, it is essential to identify the communities with the highest prevalence of DCTN and to raise awareness among the population about the importance of

an adequate diet and folic acid supplementation. Likewise, it is necessary to reinforce preventive actions and sensitize the competent authorities for the implementation of health programs with a significant impact on the morbidity and mortality of this condition and on the reduction of its disabling sequelae (12).

DEFINITION OF THE PROBLEM

How does folate deficiency influence the appearance of neural tube closure defects and how could early detection of these malformations be carried out?

OBJECTIVE

To determine the importance of folic acid and the use of biochemical and ultrasound screening strategies in the early detection of neural tube closure defects.

HISTORY

In 1964, Hibbard reported an association between malformations and folate deficiency. In 1976, Smithels et al. associated deficiency of folate and some vitamins with the recurrence of DCTN (13,14). In 1980, the results of multivitamin supplementation were published, showing a 5% recurrence rate in women who did not take supplementation, compared to 0.6% in those who did (15).

(16) suggested that women with an adequate diet would have a lower risk of recurrence. In 1981, he published a trial showing a 60% reduction in the risk of recurrence of pregnancies affected with DCTN, although the results were not statistically significant (17). Four observational studies published in the 1980s demonstrated that folic acid (FA) and multivitamin supplementation during the periconceptional period had a protective effect against DCTN (Czeizel & Dudás, 1992).

In 1991, the CDC published a review of the evidence on preventing recurrence of pregnancies affected by DCTN. In the following years, the U.S. Public Health Service recommended that all women of reproductive age consume 0.4 mg of FA per day, based on Medical Research Council trials (6). In 1992, recommendations were made that all women of reproductive age should take 0.4 mg of FA daily to reduce the risk of spina bifida and other DCTNs (7). In 1999, the Institute of Medicine reaffirmed this recommendation in its guidelines on dietary reference intake (19)

In a collaborative study between China and the USA, it was shown that FA could be effective in preventing the recurrence of DCTN at daily doses of 4 mg recommended by the PHS (5). Italian research attempted to define the frequency of pregnant women who took

PA in the periconceptional period (three months before and two months after conception). It was found that 0.1% of couples without risk had not taken PA and had offspring with anencephaly; 4.1% took PA before pregnancy; 12.3% during the first two months of pregnancy and only 0.5% in the recommended periconceptional period (20).

SCIENTIFIC NEWS

Throughout the five-year period between 2000 and 2015, the FA did not prevent all cases of DCTN. Its protective effect may be less in certain ethnic groups (8). Recent research suggests that FA may also prevent other birth defects such as cleft lip and palate, limb defects, and urinary tract abnormalities (21). Wehby et al. reported that the consumption of PA with significant protective efficacy from high doses in the periconceptional period not only prevents DCTN, but also oral clefts, which translates, on average, to a 50% decrease in recurrent events. (22). Global studies estimated that about 260,100 pregnancies per year were affected by neural tube defects (NTDs), highlighting the continued importance of FA in its prevention (23). During this same period, mandatory food fortification was confirmed as a key strategy, substantially reducing the prevalence of NTDs, although concerns persisted about the safety of unmetabolized FA (24).

While in the three-year period between 2016 and 2019, research identified that folate concentrations in erythrocytes of approximately 1000 nmol/L were optimal for the prevention of NTDs, consolidating themselves as a standard biomarker (25). At the same time, the importance of vitamin B12 together with FA was recognized, due to its close metabolic interaction, (26). In addition, inositol emerged as a possible adjunct to FA, especially for FA-resistant NTDs, although more definitive clinical studies are required (27). Other studies highlighted the positive association of FA in reducing the risk of oral cleft and congenital heart disease (28).

In the four-year period of 2020 - 2024, scientific interest in the potentially adverse effects of UMFA grew, with research suggesting tentative links with immunological and metabolic disorders, although without conclusive evidence that modified existing recommendations (29). Genetic variants of the MTHFR gene continued to be a relevant issue, although entities such as the CDC reaffirmed the position of not recommending routine screening of these variants to modify standard doses of FA (30) Mandatory fortification was implemented in 69 countries (covering 32% of the global population as of July 2023), although significant heterogeneity persists (countries with voluntary or non-existent fortification). The disparity underscores the need for expansion, particularly in low-income countries, reflecting significant heterogeneity in public policies (31). Evidence

(national studies, global meta-analyses) consistently confirms that mandatory fortification significantly reduces the prevalence of NTDs (e.g., 36-37% globally). It is estimated that its global adoption would prevent 70,000 DTNs annually. (31–34)

Finally, during 2025, there is scientific consensus on the safety of mandatory fortification at current levels. Adverse effects, association with cancer risk, decreased cytotoxicity of NK cells, links with neurodevelopment, masking of B12 deficiency are mostly considered unfounded because.the most recent research indicated possible risks of UMFA (unmetabolized folic acid), circulates when intake exceeds metabolic capacity and its detection is frequent in populations with high intake of folic acid; It is not derived from the dietary folate Natura, (35–37). Scientific opinion of the authors, When oxygen is low, tumor cells are even more dependent on folate metabolism to survive, proliferate, and defend against oxidative stress, this is how folate not only allows cancer cells to synthesize DNA at high speed, but also helps them resist oxidative damage caused by changes in oxygen availability, especially protecting against ROS toxicity. So I would not recommend Folic Acid for any patient who presents a neoplasm, however, I would do so in order to prevent mutations in the DNA that would eventually unleash a series of mechanisms until reaching cellular pleomorphism.

POSOLOGIC STANDARDIZATION

The World Health Organization (WHO) recommends 400 µg/day of folic acid for all women from the preconceptional period to the 12th week of gestation. For women with a history of NTDs (high risk), 5 mg/day, recurrence counseling, and encouragement of dietary folate intake is recommended. It promotes weekly iron and folic acid supplementation in populations with a high prevalence of anemia to address both deficiencies. (38,39)

In the United States, the Centers for Disease Control and Prevention (CDC) recommends 400 mcg/day of folic acid for all women of childbearing age, obtained through supplements and fortified foods, specifying that only synthetic folic acid has been shown to prevent NTDs. For high risk (history of NTDs), 4000 mcg (4 mg)/day is prescribed from 1 month preconception to the first trimester. It demystifies the clinical relevance of MTHFR variants for standard dosing and points to the need for targeted interventions in groups with lower folate levels (e.g., Hispanics/Latinas) (40–43). The American College of Obstetricians and Gynecologists (ACOG): Recommends 400-800 µg/day of folic acid for women of childbearing age, starting ≥4 weeks preconception. During pregnancy, she suggests multivitamins with ≥400 µg, (44–47). It indicates 4 mg/day for high risk, defining factors such as: personal/family/partner history of NTDs, type 1 diabetes, obesity, and use of

certain anticonvulsants, (48). The U.S. Preventive Services Task Force (USPSTF): Recommends (Grade A) daily supplementation with 0.4-0.8 mg (400-800 µg) of folic acid for all people planning or may become pregnant, starting ≥1 month preconception and continuing the first 2-3 months of gestation. It explicitly excludes high-risk individuals, who require individualized medical evaluation. The Grade A rating indicates high certainty of substantial net benefit, (49).

Organization	Population	Recommended Daily Dose	Specific Instructions
WHO	General	400 µg	From the attempt to conceive until the 12th week of gestation
WHO	High Risk	5 mg	Women with a history of NTD
CDC	General	400 mcg	
CDC	High Risk	4000 mcg (4 mg)	One month before conception until the end of the first trimester
ACOG	General	400-800 µg	
ACOG	High Risk	4 mg	Women with specific high-risk factors
USPSTF	General	400-800 µg	One month before conception until the end of the first trimester

SIGNALING PATHWAYS AND MOLECULAR BIOMARKERS

FRONTLINE SIGNALLING ROUTES

Neural tube defects require several signaling pathways such as Wnt/PCP, Shh, BMP, Notch, FGF which work on complex cellular behaviors necessary for neural tube closure.

WNT SIGNALING/FLAT CELL POLARITY (PCP)

The Wnt/PCP pathway is essential for the elongation and closure of the neural tube by convergent extension. (50) Precisely modulated Wnt signalling is required; mutations in components such as the LRP6 coreceptor cause defects in cranial closure. (51) Both the reduction and overactivation of Wnt signaling can cause failures in cranial closure due to interference with different cellular mechanisms, (51). LRP6 mediates canonical and non-canonical Wnt pathways (PCPs), implying its role in neurulation possibly via RhoA-dependent mechanisms, (52). In the caudal region, Lrp6-mediated Wnt/β-catenin signaling is crucial for closure, (50). Disruptions in central Wnt/PCP components cause defective convergent extension of midline neuroepithelial cells, preventing fusion of neural folds, (53) Tightly controlled Wnt signaling, involving LRP6 and interacting with canonical and noncanonical pathways, is needed for successful closure. That both insufficient and excessive Wnt activity lead to NTDs suggests a strictly regulated temporal and spatial role

(51,53). The involvement of LRP6 in both pathways implies possible crosstalk and complex regulatory mechanisms, (52).

Signaling Pathway	Key Ligands/Receptors	Major Roles in Neurulation	Examples of Associated DTNs (fragment-based)
Wnt/PCP	Wnts, Frizzled, LRP6	Neural tube lengthening by convergent extension, elevation and fusion of neural folds, regulation of cell polarity and cytoskeleton	Cranial NTDs, Craniorachischisis, Spinal NTDs
Shh	Sonic hedgehog, Smoothened, Gli	Neural tube patterning, regulation of cell proliferation and differentiation, apical constriction in lateral cells	Cranial NTDs
BMP	Bone Morphogenetic Proteins, BMPRs	Development and patterning of the dorsal neural tube, neural crest cell formation, closure of the hindbrain	Spinal NTDs
Notch	Notch receptors, DSL ligands	Regulation of neural stem cell fate, maintenance of progenitor populations, lateral inhibition	DTN (involved, but specific types not detailed)
FGF	Fibroblast Growth Factors, FGFRs	Anterior neurulation, mesoderm induction, spinal neural tube closure, rhombencephalon pattern	Anterior NTDs, Spinal NTDs

SONIC HEDGEHOG (SHH) SIGNALING

Shh is key in the closure of the neural tube, particularly in the cranial region, (53). Directs modeled cell remodeling; lateral cells drive cranial closure by coordinated apical constriction, spatially regulated by Shh, (53). The loss of Gli2 alters the cellular architecture in the midline. The absence of components of the IFT-A complex (Ift122, Ttc21b) interrupts apical constriction and the organization of actomyosin in lateral cells, failing cranial closure (54). A gradient of Gli activity mediates Shh responses in the neural tube, (55). Arachidonic acid (AA) enhances SHH-stimulated SMO ciliary enrichment (56). Dysregulation of the Hedgehog (Hh) pathway causes brain developmental defects, (55). These findings underscore distinct roles of Shh in different regions (midline vs. lateral) and stages. The Shh-cilia connection (implying IFT-A and AA) emphasizes the ciliary importance in mediating Shh during neurulation, (54).

BONE MORPHOGENETIC PROTEIN (BMP) SIGNALING

BMP regulates multiple aspects of dorsal neural tube development, (57). BMP signaling in dorsal neuroepithelial cells is critical; BMPR1A in dorsal neural folds is important for closure of the hindbrain, but dispensable for spinal neurulation (57). BMP is crucial for the patterning of dorsal cell fate and neural crest formation, (58). BMP decreases electrical activity in embryonic spinal neurons, affecting the specification of the dorsal commissural neuronal phenotype, (59). The loss of FGF3 causes elevated BMP signals,

increasing neuroepithelial proliferation and delaying closure (60). In human brain organelles, simulated microgravity (SMG) affects N-cadherin-based adherent junctions, causing NTDs associated with Hippo signaling and dysregulated BMP, (60) Computational models highlight the role of BMP in the morphogenesis of neural tube closure defects, (61) BMP has diverse and regionally specific roles, with interactions with FGF and Hippo, indicating that it is part of a complex regulatory network, (59,60).

NOTCH SIGNAGE

Notch, a conserved cell-cell communication mechanism, is significant in the development and homeostasis of tissues, including the nervous system, (62). Its results depend on the context, (62). It is necessary for the development of nervous, biliary, visual and auditory systems, (63). In the dorsal forebrain, a Notch equilibrium in progenitors is required to generate oligodendrocytes late, (64). In Drosophila, Notch regulates the termination of neurogenesis, (65). All-trans retinoic acid (atRA) promotes brain development and neural stem cell differentiation by inhibiting Notch's N1 pathway (66). It occurs by direct cell-cell communication, (67). Notch is crucial in neurodevelopment; its dysregulation could contribute to NTDs affecting neural tissue development and differentiation. Its context-dependent nature implies variable roles in neurulation, (63,67)

FIBROBLAST GROWTH FACTOR (FGF) SIGNALING

FGF has diverse roles in embryonic development, crucial in spinal cord development and regeneration, (68). Four main FGFRs and alternative splices generate seven functionally distinct receptors, (69). FGF is necessary for anterior neurulation and mesoderm induction (70). The loss of FGF3 causes elevated BMP signals, increasing neuroepithelial proliferation and delaying closure, (59)In the hindbrain, short-range Fgf participates in patterning, (71). FGF via Fgfr1 is involved in spinal neural tube closure, (72). FGF5 is overexpressed in SHH-driven childhood medulloblastoma (73). FGF is critically involved in multiple aspects of neural development (anterior and spinal), with interplay with BMP, (70,71,74).

TRANSCRIPTION FACTORS AS KEY REGULATORS

Transcription factors regulate gene expression during neural tube closure, their precise action being critical.

PAX TRANSCRIPTION FACTORS

Pax3 and Pax7 have roles in the development of the dorsal nervous system, including neural crest specification and regulation of neural tube closure, (75) In tunicates, their inactivation affects closure, suggesting an ancient role, (75). In mice, homozygous Pax3Pax8/Pax8 embryos show closure defects indistinguishable from Pax3 mutants, (76). PAX3 is expressed in multipotent precursors of neural crest and somitic mesoderm, implicating them in the etiology of NTDs, (77). Suboptimal folate levels could trigger NTDs in carriers of PAX3 mutations, (78). This underscores the conserved and crucial role of Pax3/7, with significant gene-environment interaction (PAX3-folate), (78).

ZIC TRANSCRIPTION FACTORS

The Zic family promotes proliferation and maturation of cerebellar granular neurons, (79). They are necessary for induction and repression of specific target genes, independent of changes in their binding to DNA, (79). Zic2Ku/Ku mouse embryos exhibit spina bifida due to defective BMP-dependent DLHP formation and RhoA-dependent F-actin accumulation, (80). The involvement of ZIC in closure and neural abnormalities is supported by NTDs in humans with 13q deletion syndrome (including ZIC genes), (81). A polymorphism in ZIC5 could be protective against NTDs, (81). Zic plays a role in neural closure, Zic2 in hinge formation, and cytoskeletal regulation. Variations in ZIC can modulate susceptibility to NTDs, (80,81).

GRHL TRANSCRIPTION FACTORS

Grainyhead-like factors (GRHL) are essential and conserved, regulate processes in embryogenesis and are involved in cancer, (82) GRHL2 contributes to epithelial morphogenesis, neural closure and hearing loss, (82). Grhl2 deletion in mice failure site 3 closure (exencephaly, split face), (83). The loss of Grhl3 defines a defect in the lower spinal closure, associated with defective formation of DLHPs, (83). Both the lack and increase of GRHL2 prevent spinal closure, highlighting its precise regulation, (83). Grhl3 is essential for spinal closure (null mutants with penetrating spina bifida), (84). GRHL2 and GRHL3 are critical in neural closure at different axial levels, maintaining epithelial integrity and regulating biomechanics (83–85).

TFAP2 TRANSCRIPTION FACTORS

The TFAP2 family is important in the gene regulatory network of the neural crest, (86). TFAP2A activates distinct genomic regions during neural plate boundary induction and neural crest specification, (87). Concerted inactivation of Tfap2a/Tfap2b in murine neural

crest causes medial facial cleft and skeletal abnormalities, (88). Genes such as Tfap2a induce neural crest identity when closing the tube, (89) Evolutionary studies suggest subfunctionalization between Tfap2 paralogues, (90). Although its primary role is in neural crest development, its expression at the neural plate edge and during closure suggests indirect/regulatory influence on the process (86,89).

ADHESION AND CELLULAR COMMUNICATION

Cell adhesion and communication, mediated by specific molecules and signaling pathways, are crucial for dynamic tissue remodeling of neural closure.

CADHERINAS

Fundamental in neural tube formation and integrity. During neurulation, a critical change from E-cadherin to N-cadherin, essential for neural fold fusion, occurs (91). CDH2 (N-cadherin) maintains neuroepithelial integrity, (92) Emergence of neural crest cells requires transient decrease in CDH2, (92) In C. elegans, HMR-1 (ortholog) mediates embryonic reorganization during neurulation, (93) Variants in Cadherin-11 affect cell adhesion, (93). Cadherins influence neural differentiation, (94). Simulated microgravity (SMG) affects N-cadherin-based adherent junctions, causing NTDs, (95) E-to-N switching and N-cadherin function are critical for tissue cohesion and cell rearrangements, (91,92,95).

EPH/EFHRINE

Critical for cell adhesion and repulsion, with key roles in morphogenesis, including neural closure, (96). Decreased ephrine B2 in Xenopus causes closure defects, (96). They repel retinal axons and guide cell migration, (97) Their expression in complementary domains suggests a role in tissue boundary formation, (97) FSH increases expression of Eph-ephrine limbs, (98) Unique bidirectional signaling capacity, (99) Variants in EPHA2, EPHB6, EFNB1 identified in Malaysian individuals with spina bifida, suggesting a link with NTDs, (100) Eph/ephrin signaling participates in neural shutdown (adhesion, repulsion, limits); Genetic variations can increase susceptibility, (96,97,100).

THE EPIGENETIC PANORAMA OF NEURULATION

Epigenetic mechanisms regulate gene expression without altering the DNA sequence and play a crucial role in neurulation, Neural Tube Defects and Epigenetics: Role of Histone Post-Translational Histone Modifications, (101).

DYNAMIC DNA METHYLATION

DNA methylation participates in neural development and etiology of NTDs, (101). New variants in IGF2 with altered methylation patterns are linked to DTNs, (102). Maternal folic acid status interacts with TET1 DNA demethylase to regulate brain development; Folate excess or deficiency alters hypermethylation in null Tet1 embryos, at neurodevelopmental loci (103). Folate deficiency is related to hypomethylation and genomic instability, a possible mechanism of contribution to NTDs, (104). Abnormal methylation patterns due to folic acid deficiency are consistently associated with NTDs, (101). DNA methylation regulates gene expression in neural development; alterations (genetic/environmental influence) contribute to NTD pathogenesis, (101–103)

HISTONE MODIFICATIONS AS CRUCIAL MODULATORS

Histone modifications influence gene expression and are involved in NTDs, (103). Genomic profiles of H3K27me3 and H3K27ac in a murine model of BaP-induced NTDs reveal significant alterations associated with neurodevelopmental pathways (A/P pattern, ephrin signaling, neuronal migration/differentiation) (105). NTDs can induce abnormal astrocyte development via activation and epigenetic permissiveness of STAT3 (implies histone modification), (106). Genomic alterations in H3K27me3/H3K27ac in NTDs correlate with transcriptional changes, highlighting functional impact, (101). Histone modifications are dynamically regulated; alterations (e.g., by BaP) dysregulate gene expression and contribute to NTDs, (101,105,106).

NON-CODING RNAS: EMERGING ACTORS

Non-coding RNAs (ncRNAs), such as microRNAs (miRNAs), regulate developing gene expression and are involved in NTDs, miRNAs are candidates for understanding molecular mechanisms, diagnosis and therapy, (107) Specific miRNA expression profiles are associated with NTDs (potential biomarkers), (107) Dysregulation of certain miRNAs involved in pathophysiology, (107). Maternal folate status affects differential methylation of neonatal DNA, altering gene expression (possible contribution to NTDs), (78) ncRNAs, especially miRNAs, have a regulatory role in neural development; its dysregulation is associated with NTDs, opening pathways as biomarkers and therapeutic targets, (78,107).

CELL DYNAMICS AND THE MECHANICS OF TUBE FORMATION

The formation of the neural tube depends on cellular dynamics and coordinated mechanical forces, (108).

MORPHOGENETIC MOVEMENTS AND CHANGES IN CELL SHAPE

Neural morphogenesis requires spatiotemporal coordination of changes in cell shape and position.[23] Key processes: convergent extension (tissue narrowing/lengthening) and apical constriction (apical cell contraction -> tissue folding), (108) Live imaging reveals cell dynamics, (108) Human stem cells in micropatterns show that neural and non-neural ectoderm are needed/sufficient for folding (neural apical contraction, non-neural basal adhesion), (109) In Xenopus, poor remodeling of the underlying somitic mesoderm causes defective apical constriction in neuroepithelium and closure failure, (110) Neural closure is dynamic, with coordinated cellular changes (apical constriction, convergent extension) and depends on tissue interactions (neuroepithelium-mesoderm). (108–110)

THE ROLE OF THE CYTOSKELETON

The cytoskeleton generates forces and mediates cellular changes for neural closure. Contraction of the apical actomyosin network in neuroepithelial cells contributes to folding/closure, (111). Experimental interruption (cytochalasins) causes exencephaly in rodent embryos, indicating an essential role in cranial closure, (112) SLMAP3 regulates cytoskeletal organization and PCP pathway, both crucial, (113). Prickle2 regulates apical junction remodeling and tissue fluidity, essential for epithelial deformation, (111) The cytoskeleton (actin-myosin network) is essential for cellular changes and tissue remodeling; alterations in components or regulation cause NTDs.

PRIMARY CILIA: SENSORY CENTERS IN NEURULATION

Primary cilia are important developing sensory centers, including neural formation. Their dysfunction is linked to brain malformations and neurodevelopmental disorders, (111) Involved in the etiology of neural closure defects, connecting cell fate and cytoskeletal organization, genes associated with NTDs encode ciliary proteins (e.g., IFT, for assembly/maintenance), (114) They receive environmental signals, including morphogens, (115). Ciliary-based actin remodeling/regulation impacts ciliogenesis, (116) Primary cilia are critical signaling centers; structural/functional defects (mutations) disrupt essential pathways and contribute to NTDs, (114–116).

SECONDARY NEURULATION: CONSTRUCTING THE CAUDAL NEURAL TUBE

Posterior region of the neural tube (tail) is formed by secondary neurulation, (108) It involves aggregation of mesenchymal cells (epiblast) and mesenchymal-epithelial transition

(MET) to form a tube, (108). In human embryos, it forms a single lumen (similar to mice), (117). Secondary 'splitting' is observed in proximal regions of the human tail, (117). Disruptions cause closed 'dysraphic' NTDs at lower sacral/coccygeal levels, (112). Lumbosacral myelomeningocele (medulla/meninges protrusion) is an example of an incomplete closure defect (involving secondary neurulation), Human spinal cord organoids reveal de novo lumen formation by Yap-dependent conserved cellular intercalation, (118) Secondary neurulation is mechanistically distinct; Its alteration causes caudal defects (myelomeningocele). The role of Yap/cellular intercalation clarifies molecular mechanisms, (118–120)

GENETIC AND GENOMIC PERSPECTIVES ON THE ETIOLOGY OF NTDS

The etiology of NTDs involves genetic and environmental factors, (121) Advanced genetic/genomic technologies improve understanding of genetic contributions.

UNRAVELING RISK GENES USING GWAS AND SEQUENCING

GWAS identifies genetic variants associated with NTDs via linkage imbalance, (122) Optical genome mapping (GMO) in individuals with NTDs revealed unreported structural variants and candidate genes (RMND5A, HNRNPC, FOXD4, RBBP4), (123) GMOs detected pathogenic SVs (8% cases) and variants in genes of DTN pathways (additional 13%), (123) Combined Clinical Genome-Exome Sequencing detects common (GWAS) and rare (exome) variants, Long-read sequencing identifies complex variations (useful in rare diseases), and the eMERGE Network integrates genomic and clinical data to understand disease risk.

STUDIES EXPANDING GENETIC KNOWLEDGE OF DTN

Gene Name	Type of Study	Potential Role in DTN (fragment-based)
RMND5A	GMOs	Newly identified candidate gene with strong potential for involvement in DTN
HNRNPC	GMOs	Newly identified candidate gene with strong potential for involvement in DTN
FOXD4	GMOs	Newly identified candidate gene with strong potential for involvement in DTN
RBBP4	GMOs	Newly identified candidate gene with strong potential for involvement in DTN
AMER1	GMOs	Adds risk of NTDs to known clinical implications
TGIF1	GMOs	Adds risk of NTDs to known clinical implications
IGF2	Sequencing	Genetic Variations and Altered Methylation Linked to NTDs
PAX3	Genetic Studies	Expressed by neural crest precursors and somitic mesoderm, involved in DTN, it interacts with folate levels
ZIC2	Genetic Studies	Involved in the formation of BMP-dependent DLHP and accumulation of RhoA-dependent F-actin during neurulation

GRHL2	Genetic Studies	Critical for neural tube closure at closure site 3, maintaining epithelial integrity
GRHL3	Genetic Studies	Essential for lower spinal closure and DLHP formation

THE COMPLEXITY OF GENETIC INTERACTIONS

The development of NTDs arises from complex gene-gene and gene-environment interaction, (124) Research program identified rare genetic variants (nonsense, frame-shifting, non-coding) associated with spina bifida. Investigates functional impact of variants on cellular processes (neuroepithelial polarity/proliferation) using CRISPR-Cas9 (human stem cells, mouse models), (125) Hypothesis test: folate role is to suppress RONS. It examines how genetic variants affect cellular redox state/one-carbon trafficking and whether supplements modulate effects, (125)DTN etiology involves complex gene-gene (epistasis) and gene-environment interactions, beyond the unique protective role of folate, (124,125)

THE IMPACT OF RARE GENETIC VARIANTS

Rare genetic variants contribute to DTN etiology, (125) Long-read sequencing improves identification of these alterations (crucial in rare enf., DTN subset). Rare variants (de novo, low frequency) contribute to genetic load, especially without a clear family history or when common variants (GWAS) do not explain phenotype. Their identification/characterization is crucial to understanding genetic architecture and personalized approaches, (125)

STRUCTURAL VARIATIONS AND COPY NUMBER ALTERATIONS

Structural variations (SVs: deletions, duplications, etc.) contribute to the genetic basis of NTDs. GMOs detected pathogenic SVs in individuals with NTDs, (123) NCBI dbVar is a database of SVs. Analysis/interpretation of SVs is complex; computational tools prioritize pathogenic SVs, (126). GATK-SV is a tool to call SVs, a(127)Identifying/analyzing SVs (including CNVs) is crucial to understanding genetic etiology and discovering unrecognized factors, (120,127)

FUTURE PERSPECTIVES

Recent advances have illuminated the critical roles of signaling pathways (Wnt/PCP, Shh, BMP, Notch, FGF), transcription factors (Pax, Zic, Grhl, Tfap2), and adhesion molecules (cadherins, Eph/ephrine) in neural tube closure. The role of epigenetics (DNA methylation, histone modifications, ncRNAs), influenced by environmental factors (folate), in the pathogenesis of NTDs is recognized. Studies of cell dynamics (live imaging, stem cell models) and secondary neurulation have deepened mechanistic understanding. Genomic technologies (GWAS, sequencing, GMOs) identified new candidate genes and SVs.

Gaps persist such as the precise nature of gene-gene and gene-environment interactions, and functional roles of candidate genes. There is a need to better understand the epigenetic landscape and its regulation. Future research requires advanced technologies: single-cell sequencing (cell heterogeneity), functional genomics CRISPR (gene/regulatory roles), advanced imaging (dynamic processes). Human stem cell models and organoids are valuable tools. Interdisciplinary approaches (genomics, developmental biology, environmental health) are essential for comprehensive understanding and effective prevention. It is critical to include diverse populations in genomics research to generalize findings and address health disparities.

MATERIAL AND METHODS

The present study is a descriptive review based on previous publications on neural tube development, clinical and epidemiological aspects, timely detection through prenatal screening, and primary and secondary prevention strategies for TNCD. Articles indexed in scientific databases such as PubMed, Scopus and Web of Science were included, as well as reports from international health organizations and current regulations on folic acid supplementation.

A systematic search was conducted for literature in English and Spanish languages published in the last 30 years. We included observational studies, randomised clinical trials and systematic reviews. We excluded articles without peer review and publications with insufficient data on the relationship between folic acid and the prevention of DCTN.

THEORETICAL FRAMEWORK

NEURAL TUBE FORMATION

The transformation of the embryonic general ectoderm into a thickened structure called the neural plate is the first of four stages in neural tube formation. This initial event is induced by molecular signals coming mainly from Hensen's node and the notochord,

causing significant changes in cell height and morphology, giving rise to the differentiated neuroectoderm (Sadler, 2019).

The second stage involves the progressive narrowing and lengthening of the neural plate, forming the definitive neural plate. During this process, neuroepithelial cells undergo specific regional modifications, such as increases in their cell height, mainly on the basal surface, as well as dynamic cell rearrangement (128).

In the third stage, lateral elevation of the neural plate edges occurs, resulting in the formation of a middle neural sulcus. Several mechanisms have been proposed to explain the lateral folding and subsequent closure of the neural tube. Initially, some researchers suggested a single dominant mechanism, but later research indicates that lateral folding results from multiple specific mechanisms both intrinsic and extrinsic to the neural plate. The medial ventral region of the neural plate, also known as the hinge midpoint, acts as a pivot where lateral curvature is carried out thanks to apical contraction mediated by actin microfilaments located at the apical end of neuroepithelial cells, thus allowing the formation of neural tube closure (129).

These neuroepithelial cells undergo a basal narrowing of the nucleus, resulting in lateral expansion and contraction similar to a closing mechanism (such as a purse rope) thanks to the aforementioned actin ring. This mechanism is reinforced by extrinsic forces coming from the lateral epithelium to the neural plate, contributing to the complete closure of the neural tube.

Fig.1

Early Stages of Central Nervous System Formation

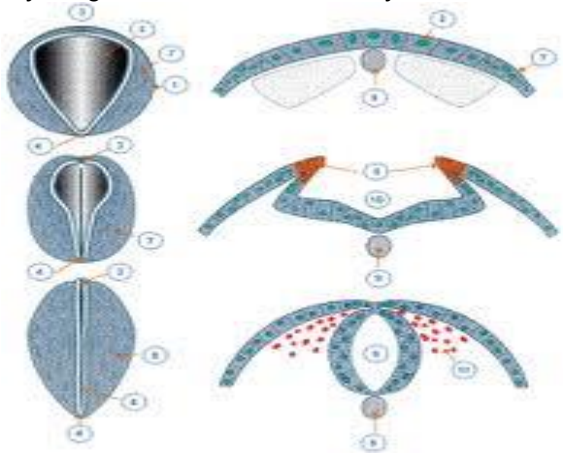
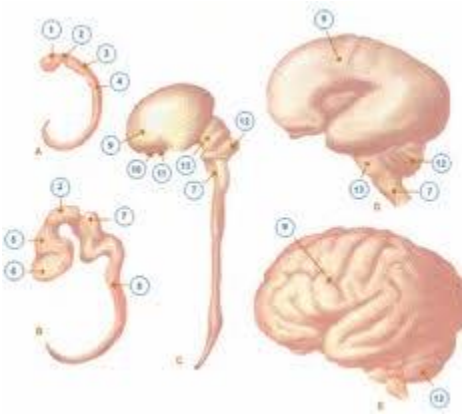


Fig. 2

Diagram of the Cavities of the Spinal Cord and Brain Vesicles



These cells begin to narrow from the basal position of the nucleus, causing a lateral expansion of the cell in that area, accompanied by a contraction similar to the closing mechanism of a purse, mediated by an actin ring. This ring contains microfilaments located at the apical end along the entire neural plate fold and in the spinal cord region, thus facilitating effective closure of the neural tube. Many adjacent structures are flattened due to this process. The elevation of neural folds is complemented by extrinsic factors generated from the surface of the lateral epithelium adjacent to the neural plate.

The fourth stage in neural tube formation involves apposition and fusion of the apical-lateral surfaces of the neural folds, ending with complete separation of the neural tube from the underlying ectoderm. At the same time, neural crest cells separate and migrate from the neural tube. According to (130), neurulation is regulated by two genetically organized switches: one specifically responsible for the formation of neural tissue and the other related to neural specification. This hypothesis underlines that cell adhesion and movement are controlled by these switches.

The main early morphological response of the embryonic ectoderm to primary induction, mediated by cellular and molecular interactions generated by Hensen's node organizer and the notochord (128), consists of an increase in the height of the cells destined for the nervous system, which produces a visible thickening called neural plate on the dorsal surface of the early embryo. It is important to highlight the differential restriction in the expression of cellular adhesive molecules (CAMs). Specifically, N-CAM and L-CAM coexist in the pre-induced ectoderm; however, after primary induction, cells destined for the neural plate retain N-CAM expression while losing L-CAM expression, the exact opposite occurring in the non-neural ectoderm, which expresses L-CAM and loses N-CAM (128).

Neural tube closure begins in the middle region of the craniocaudal extension of the nervous system approximately on days 21-22 of embryonic development, at the level of the fourth somite. From this central point, the closure progresses in the cranial and caudal direction, extending in a similar way to the closure mechanism of a zipper. The cranial and

caudal openings that remain temporarily unclosed are called anterior and posterior neuropores respectively. The posterior neuropore normally closes around day 27 after conception. When these neuropores fail to close completely, they can lead to severe birth defects (128).

Caudally to the posterior neuropore, in animals with prominent tails, the remnant of the neural tube is formed by secondary neurulation. This mammalian process involves the formation of a rod-shaped mesenchymal condensation under the dorsal ectoderm of the caudal bud. Within this condensation, a central cavitation channel is generated, which is continuous with the neural tube formed during primary neurulation. Although this process is not prominent in humans due to poor development of caudal spurt, (131) axial curvature is an essential factor in neural tube closure, thus constituting a crucial aspect in neurulation.

NEURAL TUBE SEGMENTATION

Once the neural tube (NT) takes shape, the region destined for the brain begins to be clearly distinguishable from the rest that will form the spinal cord. This early brain area undergoes sequential subdivisions that lay the structural and functional foundation for the adult brain. Initially, the brain is divided into three main segments: the forebrain (forebrain), midbrain (midbrain), and hindbrain (hindbrain) (128).

From the fifth postconception week, the forebrain is segmented into two specific regions: the telencephalon or terminal brain, which will originate the primitive cerebral hemispheres, and the diencephalon or intermediate brain, from which the optic vesicles will emerge through evaginations from the forebrain itself. Other evaginations of the diencephalon will give rise to glandular structures such as the pineal gland and the anterior pituitary gland (128).

At the same time, at the same stage of embryonic development, the hindbrain also divides into two fundamental parts: the metencephalon, which will give rise to the cerebellum and pons, and the myelencephalon, which will form the medulla oblongata. On obstetric ultrasound, the rhombencephalon can be identified as a clearly defined cystic image at the head pole, usually described as a monoventricular image between 6-8 weeks post-conception, equivalent to 8-10 weeks of amenorrhea (128). It should be noted that the rapid growth and dorsal rotation of the cerebral hemispheres derived from the telencephalon generate a midline confluence with the diencephalon and midbrain (128)

In addition to this traditional segmentation, there is another form of transient segmentation in brain development, initially described more than 150 years ago by early embryological studies. This secondary segmentation, visible in the NT hindbrain in several

vertebrate embryos studied, is known as neuromers. Although their significance has been debated, these structures are transiently observable in human embryos between the fourth and fifth late week. Despite their short duration, neuromeres provide fundamental organizational foundations for the development of the nervous system. Studies in chicken embryos have shown that the cell bodies of certain cranial nerves come specifically from definite neuromeres (128,129,131–133).

The appearance of neuromeres originates through individualized cell proliferation centers within the neural tube. This significant proliferation can be attributed to local areas with high mitotic activity. Once formed, neuromeres exhibit cellular behavior similar to that observed in insect embryos, where adjacent neuromer cells do not mix. However, the specific mechanisms that control this cell segregation in vertebrates are not yet fully clarified.

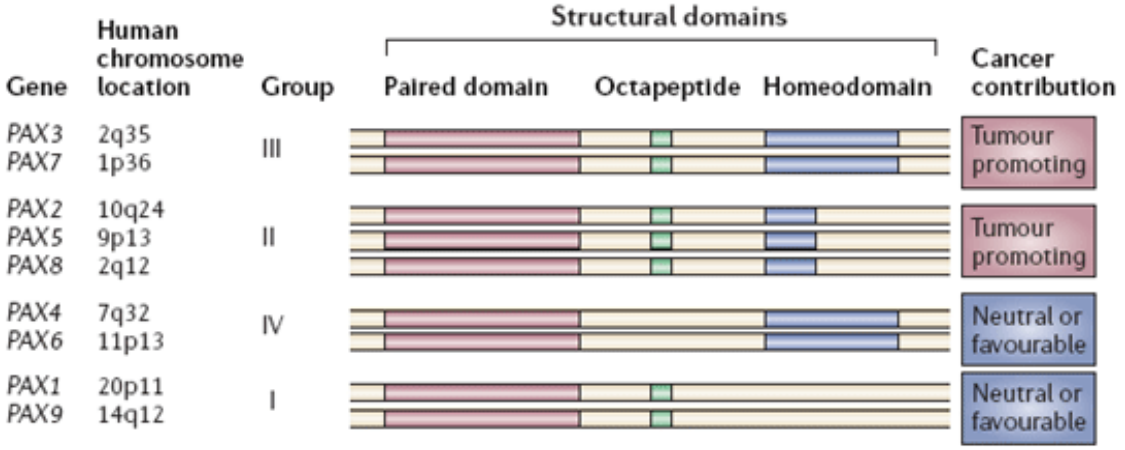
Recent studies on homeobox gene expression in vertebrates have identified a precise correlation between the distribution of specific gene products and individual neuromers. Homeobox genes are phylogenetically conserved, characterized by a nucleotide sequence of 183 bases, encoding a protein domain (homeodomain) of 61 amino acids capable of binding to DNA and acting as transcriptional factors (128). Among these genes, specific members such as the Hox-2 cluster (Hox-b), Krox-20, Wnt and engrailed, functionally related to the segmentation genes observed in Drosophila, stand out.

These homeotic genes play an essential role in specifying the identity of neuromers and determining their downstream derivatives. Although neuromeres are not observed in the region of the neural tube corresponding to the spinal cord, there is a clear segmental pattern evidenced by the arrangement of the motor and sensory roots in this area, reflecting an underlying segmental organization.

Additionally, specific genes such as Pax-3 and Pax-7 also show restricted segmental expression. The Pax-3 gene is expressed in both the neural tube and the paraxial mesoderm and is crucial for the activation of the myogenic determinant factor MyoD. On the other hand, the Pax7 gene is predominantly expressed in the dorsal region of the neural tube and in adjacent somites. Both genes belong to the Pax family, characterized by having a paired "box" domain involved in critical developmental processes (Sadler, 2019).

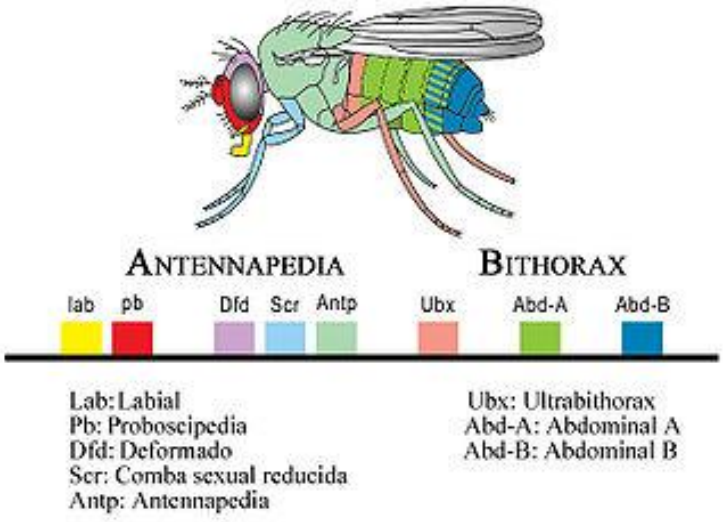
Recent studies such as (134,135) have suggested an important association between reduction in telomere length and greater susceptibility to neural tube closure defects, thus highlighting the importance of additional molecular factors in the correct execution of the neurulation process.

Fig. 3
PAX Gene Structure



Source: Nat. Rev. Cancer. 2006

Fig. 4
Metameric structure of Drosophila



Source: Nat. Rev. Cancer. 2006

Joosten and Hol suggested a functional relationship between the PDGFR alpha gene (PLATELED-derived growth factor receptor-alpha) and Pax1, establishing an activated transcriptional factor of the PDGFR alpha gene (136).

GENES RELATED TO FOLATE METABOLISM

Folate is an essential component in nucleotide synthesis and DNA methylation. Alterations in genes involved in your metabolism may increase your risk of neural tube defects (NTDs).

MTHFR (Methylenetetrahydrofolate reductase): Polymorphisms such as C677T and A1298C in the MTHFR gene have been associated with an increased risk of NTDs due to a reduction in enzyme activity, which affects the availability of active folate (137,138). The Wnt/PCP pathway is crucial for cellular movements that allow proper closure of the neural tube. VANGL1 and VANGL2 (VANGL Planar Cell Polarity Proteins 1 and 2): Genes involved in the regulation of planar cell polarity. Mutations in these genes have been associated with NTDs in human and animal models (139,140). Cilia play a crucial role in signaling during embryonic development. SHH (Sonic Hedgehog): Key ligand in Hedgehog signaling. Alterations in the SHH pathway can affect cilia formation and function, contributing to NTD (56,141). These genes encode transcription factors essential in neural development. ZIC3: Mutations in ZIC3 have been associated with heterotaxy and neural tube defects, including spina bifida(142,143). ZIC5: Participates in the formation of the neural crest and the closure of the neural tube. Alterations in ZIC5 have been related to NTDs in animal models (144).

These transcription factors are involved in the formation and maintenance of epithelial tissues.

GRHL3: Participates in the formation of the epidermis and the closure of the neural tube. Alterations in GRHL3 have been linked to spina bifida and other NTDs (145). SDC4 (Sindecan-4): Heparan sulfate proteoglycan that regulates cell migration during gastrulation. In Xenopus laevis, SDC4 disruption affects convergence and extension movements, essential for neural tube closure (146)

PREVALENCE

Neural tube closure defects (NTCD) have a remarkably high prevalence within the group of congenital malformations, although this prevalence can vary considerably depending on the specific population, geographic location, time period, and maternal demographic characteristics. In the United States, a prevalence at birth ranging from 4 to 10 cases per 10,000 live births has been reported (147). Countries such as Ireland, the United Kingdom, northern China, Hungary, and Mexico have shown significantly higher prevalences, approximately 1% of the total population, which could reflect true epidemiological differences or simply variability attributable to the prenatal diagnostic methodologies used, including earlier diagnoses and subsequent terminations of pregnancy (148).

With respect to gender differences, anencephaly occurs more frequently in female fetuses, establishing a ratio of approximately 2.3:1. On the other hand, spina bifida also shows a significantly higher prevalence in female fetuses (149)

In terms of ethnic differences, in the United States, the prevalence of DCTN among black individuals is significantly lower compared to the white population, while the Spanish-speaking population has the highest rates. Populations in western and southern Africa, as well as those in other regions of Africa, the Caribbean, North America, Europe, China, and Singapore, tend to report lower rates of (149,150).

The risk of recurrence for a second pregnancy with a previous history of TNCD is considerably higher than the general population risk, estimated in a range of 3% to 5%, depending on the population studied (148). Hendricks specifically reported that among Mexican Hispanic women, the risk was 15.1 per 10,000 live births, while for U.S.-born Hispanics this risk decreased to 9.5 per 10,000 (148,151)

Studies such as that of Bianca S. (152) revealed important epidemiological associations between the previous occurrence of spontaneous abortions and the subsequent prenatal diagnosis of congenital cardiovascular malformations (CVMC) and

TNCD. This study demonstrated a higher frequency of miscarriages in previous pregnancies in women with fetuses affected by MCVC (28.7%) and DCTN (25.1%), compared to other types of congenital malformations (8.6%), these differences being statistically significant ($p<0.05$).

Based on the RENAC 2022 Annual Report, which presents the results for the year 2021, a total of 504 cases of neural tube defects (NTCD) were registered in Argentina, which is equivalent to a prevalence of 7.9 per 10,000 live births. This rate, although consistent with national historical records, continues to be a priority indicator of epidemiological surveillance due to its impact on infant morbidity and mortality. The data were collected in more than 300 maternity hospitals throughout the country, which reflects a representative population coverage and reinforces the need to maintain prevention strategies such as food fortification with folic acid, as well as to improve prenatal screening and follow-up of at-risk pregnancies (153)

ETIOLOGY

ETIOLOGY OF NEURAL TUBE CLOSURE DEFECTS (NCD)

Despite multiple investigations conducted over the past few decades, the exact etiology of neural tube closure defects (NTD) still remains uncertain. Various clinical series have shown that these defects occur more frequently in women, particularly in the case of spina bifida, with an estimated male:female ratio of approximately 1:1.3. In the case of anencephaly, this ratio increases to 1:3, except in regions with a low frequency at birth, such as the United States, where the relative risk is estimated to be around 1:1 (148).

It has been widely demonstrated that the etiology of DCTN is multifactorial, involving both genetic predisposition and environmental factors. Numerous genes appear to be involved in this condition, and a critical genetic threshold is considered to exist whose interaction with environmental factors during a specific period of embryonic development, known as the teratogenic period, significantly increases the risk of these malformations (154).

Certain cases of DCTN are also associated with specific chromosomal abnormalities, such as trisomy 18, or clearly defined singular genetic defects, e.g., Meckel Gruber syndrome, characterized by occipital encephalocele, polycystic kidney, and polydactyly (155).

However, most cases of DCTN are sporadic and multifactorial in origin, resulting from complex interactions between genetics and environment.

The research work of Dr. Farag and his team proposed a hypothesis according to which certain congenital anomalies in organs such as the heart, spleen, kidney, intestine and limbs could originate as a result of overdistension of the embryonic neural tube. This hypothesis, in addition to providing a plausible explanation for the phenotypic diversity observed under these conditions, offers a practical approach that can be validated in future experimental and clinical studies (156).

TABLE 1. Prevalence of DCTN in Maternidades Argentinas 1992

Hospital	Delivery	DCTN	%
Castex	3460	2	0.58
Sardinian	6773	12	1.48
Argerish	1872	3	1.60
Posadas	3147	6	1.91
Gonnet	1983	4	2.02
Lazy	2882	6	2.08
St. Rose	2477	6	2.42
Clinical	1131	4	3.54
Fernandez	2243	10	4.46
Rivadavia	1886	10	5.30
TOTAL	**27854**	**63**	**2.54**

RISK FACTORS

GENETIC FACTORS. - All observations, especially family studies, ethnic differences that persist after immigration; the effects of parental consanguinity suggest that DCTNs occur within a pattern of inheritance, similar to other malformations (Table 3)

TABLE 2

Factors that have been implicated in the appearance of D.C.T.N.

Polygenic/multifunctional inheritance (13,22,29,47)	Genetic or Mendelian defects (13,22,29,47)	Chromosomal abnormalities (13,13,22,29,47)
Geographical factors Racial influence Seasonal influence Twins Risk of recurrence Sex Maternal age Economic level Phytophtora infestans (potato fungus)	Roberts syndrome Fraser syndrome Craniolencephalic dysplasia Warbug syndrome Sacred defects Frontofasionasal dysplasia Kousseff syndrome Meckel-Gruber syndrome Thrombocytopenia with absence of radius (ART syndrome) MTHFR	Trisomy 13 Dup (7p) Trisomy 18 Dup (8q) Trisomy 21 Dup (11q) Trisomy 14 Dup (22)(pter>q11) Trisomy 9 Dup (13q) Triploidy Dup (13)(Pter-q14) From (2q) r(13) Dup(2q) r(22) Dup(3q) del Xp Dup (6q)

Autosomal recessive inheritance is not likely because the proportion of affected siblings is very small from the expected 25%, and because affected adults have a relative risk of 4-5% of affected children. With dominant inheritance, penetrance would tend to be very low and cytoplasmic inheritance is very likely, because affected mothers do not have a greater risk of having affected offspring

TABLE 3

Familial pattern of spina bifida and anencephaly compared to other common malformations (22)

	Cleft lip (+/- cleft palate (London)	Bot foot (Exeter)	Spina Bifida and anencephaly (London)	Pyloric stenosis (men) (London)	
Frec Nacimiento* Family Patron** Related Related Related	1 X 40 X7 X 3	1.2 X 30 X 5 X 2	2 X 15 - X 2	X 25 X 9 X 1 1/2	7.7 X 7 - X 1 1/2

ROLE OF FOLATE AND GENETIC FACTORS IN NEURAL TUBE CLOSURE DEFECTS

During fetal development, nucleic acid and protein synthesis reaches peak levels, during which time folate availability may be insufficient. This limitation leads to inhibition of nucleic acid synthesis, negatively affecting cell replication due to insufficient DNA production during mitosis. Researchers have suggested that primary dietary deficiencies or inborn errors in folate metabolism could explain this pathogenic mechanism, thus justifying why folic acid (FA) administration has preventive effects on the development of neural tube closure defects (NTCD). According to this hypothesis, the fetus may be folate deficient despite apparently adequate maternal levels (148,157–161)

(162) have reported that polymorphism in the gene encoding the enzyme methylene tetrahydrofolate reductase (MTHFR) is an important genetic risk factor for the development of DCTN. In addition to this, another relevant gene identified is methionine synthetase (MTR). It was observed that 20% of cases and 18% of affected mothers were homozygous for the MTHFR variant, compared to only 11% in healthy controls. This genetic condition greatly increases the risk of DCTN, especially if the mutated genotype is present in mother and child simultaneously. It was also evidenced that polymorphism in the MTR gene is associated with an increased risk when folate levels in red blood cells are decreased, highlighting an important genetic-nutritional interaction (148,154,157–159,162)

Recently, (163) they identified a thermolabile variant of the MTHFR gene as a specific cause of folate-dependent DCTNs, strengthening the hypothesis of the impact of the genetic-nutritional interaction.

(164) established that the Pax3 gene mutation can also cause DCTN, by interfering with early muscle development. This study determined that Pax3 is crucial for the activation of the Denis myogenic determining factor (MyoD).

In the final analysis after evaluation by (134,135) they demonstrated in animal models that neural tube closure defects can originate from telomere shortening, resulting from the loss of telomerase function, thus highlighting another relevant molecular factor in the etiology of these congenital defects.

ENVIRONMENTAL FACTORS

ENVIRONMENTAL AND SOCIOECONOMIC RISK FACTORS FOR NEURAL TUBE CLOSURE DEFECTS (NDCT)

Experimentally, a wide range of environmental aggressions related to the appearance of neural tube closure defects (NTCD) have been observed, including vitamin deficiencies or excesses (157,161,165–167). Martínez-Frías, in a Spanish collaborative study, analyzed congenital malformations associated with the consumption of illegal drugs, identifying significant increases for DCTN, choanal atresia, esophageal atresia, gastroschisis, anal atresia and postaxial polydactyly (154,168).

In turn, it has been experimentally demonstrated that the administration of exogenous retinoic acid can induce defects of the anterior neural tube (anencephaly) and posterior neural tube (spina bifida), depending on the genetic susceptibility of the experimental models in rats (154).

Among the socioeconomic and environmental risk factors associated with DCTN are drinking water quality, influenza infections, maternal obesity, and heat exposure. (169) specifically investigated the relationship between maternal fever and DCTN, concluding that women with febrile episodes of 38.9°C or higher during the first month of pregnancy have an increased risk of developing these defects.

In addition, certain parental occupations have also been studied as potential risk factors. Lin et al. attempted to establish the association between maternal physical labor during the periconceptional period with DCTN and cleft lip, although the results did not reveal a conclusive association, suggesting the need to improve methods for measuring exposure and adequately subdividing defect types for future studies (154).

In epidemiological terms:

1. United Kingdom

   In Scotland, a study covering 2000 to 2021 reported a total prevalence of NTDs at birth of 9.8 per 10,000 total births (95% CI 9.2, 10.4), with a specific prevalence of spina bifida of 5.80 per 10,000 births (170). Notably, no significant change in the prevalence of spina bifida was observed during this period (RPP 0.99, 95% CI 0.98, 1.01, p=0.35) (170) Data from England between 2000 and 2019 indicated a prevalence of NTDs of 12.5 per 10,000 total births (95% CI 12.1 to 12.9), with 2127

cases of spina bifida reported (171). A more recent figure suggests that open spina bifida occurs in about 6 out of every 10,000 births (0.06%) in the UK (172,173).

2. United States

   In the United States, spina bifida affects approximately 1 in 2,875 births annually (174). Data from 2020 indicate that approximately 1,278 babies are born with spina bifida each year (174). There are significant disparities between racial and ethnic groups, with Hispanic women exhibiting the highest prevalence at 3.80 per 10,000 live births, followed by non-Hispanic white women at 3.09, and non-Hispanic black or African American women at 2.73 per 10,000 live births (175,176).

3. Canada

   In Canada, approximately 120 to 150 fetuses are affected by spina bifida each year, with an overall rate of about 2.6 per 10,000 births (177,178). Data from 2006 to 2020 indicate a stable trend in neural tube defects, with a prevalence of 4.8 cases per 10,000 total births (179–181). There are regional variations, with Newfoundland and Labrador reporting a rate of 3.94 per 10,000 total births in 2020 (179,182,183).

4. Argentina

   The proportion of fetal deaths due to DCTN was 1.32 during the period from 1994 to 2019, (Bronberg et al., 2023). The birth prevalence of isolated cases of DCTN reported to the National Registry of Congenital Anomalies of Argentina (RENAC) between November 2009 and December 2013 was 7.4 per 10,000 births (95% CI: 6.7–8.0), (185–187) The prevalence at the national level was significantly higher in public hospitals (9.72 per 10,000 births) compared to private/social security hospitals (6.48 per 10,000 births) during the period covered between October 2010 and December 2018.), (188).

5. China

   China has one of the highest DCTN prevalence rates in the world, (189,190). A recent systematic survey found that the perinatal prevalence of birth defects in China could be as high as 208.94 per 10,000 in 2020-21, (191). In Shanxi Province, the prevalence of DCTN was 20.09 per 10,000 births between 2017 and 2022, making it the most prevalent birth defect in the region, (192). However, a substantial decrease in the prevalence of DCTN was observed in Shanxi Province between 2003 and 2022, with rates decreasing from 116.75 per 10,000 in 2003 to 18.80 per 10,000 in 2022, (193). Whereas, in Jinan, a distinct increase in the incidence of birth defects was observed between 2005 and 2022, with DCTN being one of the most prevalent, (194). One study reported a prevalence of DCTN of 3.6%, (195). The incidence of

DCTN in Shaanxi Province is predicted to continue to decline, with projected rates of 0.49/10,000 in 2023, 0.41/10,000 in 2024, and 0.35/10,000 in 2025, (196).

6. Ecuador

   While equatorial epidemiology offers a vast wealth of statistical data, this compilation, consistent with its focus on country analysis, is limited in terms of the information available on spina bifida in Ecuador for the period 2020-2025, given the scarcity of relevant research fragments. However, a study for the five-year period 2015-2019 is available, which will provide valuable context for understanding the previous situation and possible trends. It showed a cumulative incidence of congenital anomalies of 20.89 per 1000 live births (197). Congenital malformations of the nervous system, including spina bifida, accounted for 12.21% of these cases (197). Regional variations in the incidence of congenital anomalies were observed in different cantons and provinces (197).

7. Mexico

   Populations of Mexican descent exhibit a high occurrence of neural tube defects (198). Data from U.S. birth defects registries reported a prevalence of spina bifida at birth of 3.8 per 10,000 Hispanic American live births, compared to 3.09 per 10,000 non-Hispanic white live births (198) This disparity persists despite folic acid fortification efforts, suggesting the influence of other factors such as acculturation and dietary habits (199)

8. India

   The prevalence of NTDs in India shows significant regional variation. A population survey in rural eastern Uttar Pradesh reported an incidence of 7.48 per 1000 live births (200). A systematic review and meta-analysis of studies up to 2023 found an overall NTD prevalence of 9.46 per 1000 births (95% confidence interval from 8.01 to 10.91), highlighting an increasing trend in recent decades (201). Spina bifida was reported as the second most common NTD in a 2013 systematic review, with a prevalence of 1.9 per 1000 births (202).

9. South Africa

   The prevalence of NTDs at birth in South Africa is estimated to be 8 to 12 per 10,000 live births after mandatory fortification of wheat flour with folic acid (203). Spina bifida alone affects approximately 350 live infants per year (203). While fortification has led to a 30% decrease in the prevalence of NTDs at birth, challenges remain, such as the exclusion of cake flour from mandatory fortification (203).

10. Brazil

In Brazil, the estimated prevalence of spina bifida is 14 cases per 10,000 births, and the total of all NTDs is 24 per 10,000 births (de Souza et al., 2011). A study comparing the pre- and post-folic acid fortification periods (1999-2004 vs. 2005-2010) showed a rate ratio of 1.05 for spina bifida, with a further increase to 1.4 when comparing 2005-2010 and 2011-2020, suggesting a possible increase in spina bifida rates after fortification (Vieira et al., 2022). However, another study reported a decrease in the overall prevalence of NTDs from 0.79 per 1000 before fortification to 0.55 per 1000 after fortification (2005-2014) (Gomes et al., 2024).

Table 1: Prevalence of Spina Bifida and Neural Tube Defects (NTDs) in Selected Countries (2020-2025)

Country	Prevalence of NTDs (per 10,000 births)	Prevalence of Spina Bifida (per 10,000 births)	Year(s)	Fragment ID(s)
Scotland (United Kingdom)	9.8	5.80	2000-2021	1
England (United Kingdom)	12.5	N/A	2000-2019	4
United Kingdom (SB Open)	N/A	6 (0.06%)	2020-2025	6
United States	N/A	3.5 (1 in 2,875)	2020-2025	8
Canada	4.8	2.6 (120-150 cases/year)	2006-2020	11
Australia	4.6 (births 1998–2005)	0.6 (150 cases/year)	2020-2025	20
China (Shanxi)	20.09 (2017-2022)	N/A	2017-2022	23
Ecuador	N/A	N/A	2015-2019	24
Mexico (U.S.)	N/A	3.8 (Hispanic)	2020-2025	25
India	9.46 (combined, through 2023)	1.9 (2013 Hotfix)	Until 2023	28
South Africa	8-12	3.5 (350 live births/year)	2020-2025	31
Brazil	24 (DTN est.)	14 (est.)	2020-2025	32

Note: Prevalence rates may vary depending on the specific study, population, and time period. N/A indicates that no specific data on the prevalence of spina bifida were available in the research fragments for that country, or vice versa for the prevalence of NTDs.

Pharmacologically, aminopterin, a potent folic acid antagonist historically used as an abortifacient, is the only drug with proven evidence of predisposing to DCTN. Although steroid hormones (including oral contraceptives) and antiepileptic drugs, especially valproic acid, are also suspected to increase the risk, there is no definitive evidence to confirm this yet. However, (204) they demonstrated in experimental models that valproic acid analogues induce DCTN and affect cell proliferation, increasing the incidence of these defects.

Contamination of drinking water with trihalomethanes has also been associated in isolated cases with DCTN, although other contaminants, such as haloacetic acids, have not shown a significant relationship (162).

Regarding nutritional factors, recent studies have indicated that women with children affected by DCTN generally have diets deficient in essential nutrients during the first trimester of pregnancy, compared to mothers whose children do not have defects. These observations have led to the recommendation of preconceptional dietary testing as part of antenatal counseling. In particular, folate, a water-soluble B vitamin, is crucial due to its role in DNA and RNA synthesis and in the conversion of homocysteine to methionine. Folate deficiency is clearly associated with the occurrence of DCTN, and preconceptional folic acid supplementation has been shown to significantly reduce the risk of recurrence (154,157,160,162,205).

In addition, elevated plasma homocysteine levels also significantly increase the risk of DCTN. Recent studies suggest that folic acid administration may prevent such an increase. Lakshmi et al. specifically investigated the role of pyridoxine and riboflavin in homocysteine metabolism, noting that pyridoxine effectively reduces their plasma levels, while riboflavin shows no significant effects (206).

A major meta-analysis (Huang et al., 2017) confirms that maternal obesity is a clear and significant risk factor for neural tube defects in the infant. The risk increases the higher the mother's Body Mass Index (BMI), especially above 30, (207).

CLINICAL ASPECTS

Epidemiological and familial studies are based on the fact that major neural tube defects are caused by a failure to close the neural tube at the end of the 4th week of development. They are all interrelated and probably have the same multifactorial etiology. When the closure defect occurs at the cranial end of the neuroaxis, it is anencephalic. This is a lethal condition, represented by a mass of lethal congested neural tissue, represented by a mass of congested and disorganized neural tissue, leading to the expansion of the skull. Some cases are associated with rachichis, in which all or part of the caudal end of the neural tube fails to close, or there may be a discrete cystic spina bifida.

A lower DCTN of the neuroaxis results in an encephaloid, a relatively uncommon lesion, in which a posterior defect of the skull or upper cervical spine allows the meninges to protrude through the calota, almost always covered with skin or a thick membrane. Such patients can survive with minor paralysis; But in some cases children develop severe mental retardation. When the closure defect occurs in the lower portion of the stem: you

develop cystic spina bifida. This is a highly variable condition, with some being born with a portion of the spinal cord exposed on the surface like a neural plate, with an open myelocele or myelomeningocele. Some cases have associated deformity at the base of the skull (the Arnold-Chiari malformation). Which causes hydrocephalus, present at birth in 80% of cases. This is progressive and leads to major clinical problems. Disruption of the spinal cord at the site of the defect causes paralysis of the leg, urinary and fecal incontinence, skin anesthesia, and abnormalities of the hip, knee, and feet. These cases have a poor prognosis and if left untreated, it leads to meningitis and hydrocephalus. In some cases the spinal injury may be closed and covered by a thick membrane or skin, resulting in a better prognosis: one case in every 20 cystic spines bifida is a true meningocele, with a long and intact neuroaxis. The defect is confined to skeletal tissue, allowing the meninges, covered by a thin membrane, or skin, to protrude through the defective vertebral arches. These children have little or no neurological or intellectual deficits. These types of malformations occur in some particular cases and are determined by the precise time of the closure failure. Spina bifida occulta is the simplest variety and involves only one vertebral arch, and is part of the normal variation that probably have no clinical expression. Out of every 50 hidden bifid spines, one belongs to the complicated variety. From this it follows that spina bifida involves more than one vertebral arch that is frequently associated with canal width, vertebral body abnormalities, a tuft of hair or nevus; foot deformity or some neurological deficits. Family and radiological studies showed that this variety is part of the spina bifida/anencephalic syndrome. At the same time, they showed that hydrocephalus not associated with cystic spina bifida is not part of the malformation complex of DCTNs, except in some isolated cases. The same occurs in DCTNs that are part of other malformation complexes, such as chromosomal abnormalities or Sind. Meckel-Gruber (occipital encephalocele, polycystic kidney and polydactyly), the latter with Autosomal Recessive inheritance.

I will try to summarize the classification of DCTNs in Table 4 and then I will make a description of each of them.

TABLE 4 **CLASSIFICATIONS OF THE D.C.T.N.**

Craniorachychisis Anencephaly
Holocrain (defect that reaches the foramen magnum)
Merocrain (defect does not reach foramen magnum)
Encephalocele
Occipital
Parietal
Frontal
Nasopharyngeal
Iniencephaly
Spina Bifida

Open Meningocele Myelomeningocele Myelocele Hidden Arnold-Chiari malformation Dandy-Walker syndrome Agenesis of the corpus callosum

ANENCEPHALY

Anencephaly represents the most serious and frequent defect within open neural tube abnormalities and is the most common abnormality affecting the central nervous system (CNS). Epidemiologically, it shows a marked female predominance, with an approximate ratio of 4:1 in relation to males.

Clinically, anencephaly is characterized by the partial or complete absence of brain tissue and the cranial vault, with acrania constantly being found. The cranial bones present are usually membranous, while the cranial base may retain cartilaginous structures, including orbits.

There are multiple hypotheses about the pathogenesis of this malformation:

- The most common neural tube defect (NTD) is anencephaly. Overall, the estimated prevalence of anencephaly is 3 per 10,000 births, although the incidence varies among different geographic regions, ethnic groups, and environmental exposures, (208).
- Another theory proposes that the absence of bone covering originates due to secondary destruction, either by mechanical or chemical agents during the early formation of the embryo, causing anencephaly as a final result (166).
- An association between anencephaly and osteogenesis imperfecta has been described, supported by ultrasonographic findings specific to the fetal brain, which suggest the possibility of structural damage prior to or simultaneous to neural development (166).

Wilkins-Haug et al. conducted a longitudinal study using serial prenatal ultrasonography that confirmed in humans a process similar to that described in experimental animals, observing the progression from an initial intermediate stage (exencephaly) to defined anencephaly, which contributes to validating these pathogenic theories (209).

ULTRASOUND DIAGNOSIS AND CLINICAL CHARACTERISTICS OF ANENCEPHALY

Anencephaly can be detected regularly by obstetric ultrasound, especially during the measurement of biparietal diameter to determine gestational age. In addition, early identification is becoming more and more frequent due to the incidental finding of elevated maternal alpha-fetoprotein (AFP) levels in routine prenatal screening programs.

Ultrasonographically, the absence of the bones of the cranial vault allows a specific diagnosis of anencephaly to be established. This feature is clearly visualized in coronal images of the fetal face, although sagittal images can also be informative. It is crucial to verify that the absence of the shell is symmetrical, since the cranial base and orbits are usually present, which can induce diagnostic errors if they are not properly evaluated.

In 40-50% of cases of anencephaly, the presence of polyhydramnios is reported, although this phenomenon generally appears after 26 weeks of gestation. Occasionally, oligoamnion may also be observed.

It is important to distinguish anencephaly from severe microcephaly, as both conditions can look similar on ultrasound. In cases of microcephaly, the cranial vault is always present, allowing for clear differentiation.

A rare entity known as a holocardial acephalic twin may present as an extreme anencephaly aberration in monozygotic twins. This anomaly involves the almost complete absence of cranial structures or the presence of severe microcephaly, with one twin being completely dependent on the other (parasitic twin).

Anencephaly is a lethal congenital malformation, characterized by the significant absence of most of the brain and the lack of cranial bony covering. Although portions of the forebrain may remain, the cerebral hemispheres and parts of the midbrain are usually absent. The spinal cord is usually morphophysiologically normal, except in severe cases of craniorachischisis.

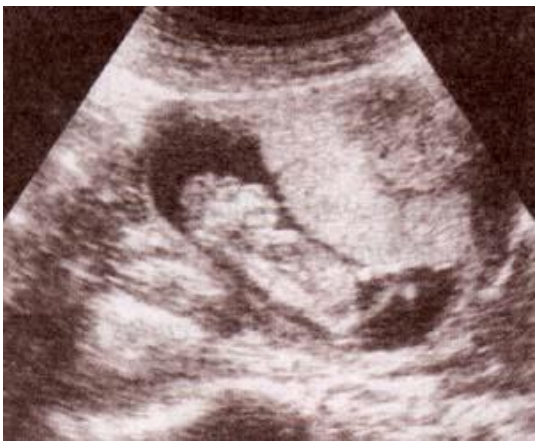
The prevalence of anencephaly varies considerably by geographic, racial, and sexual factors, with an estimated 1 per 1,000 births in the United States, and up to 6.7 per 1,000 births in Britain.

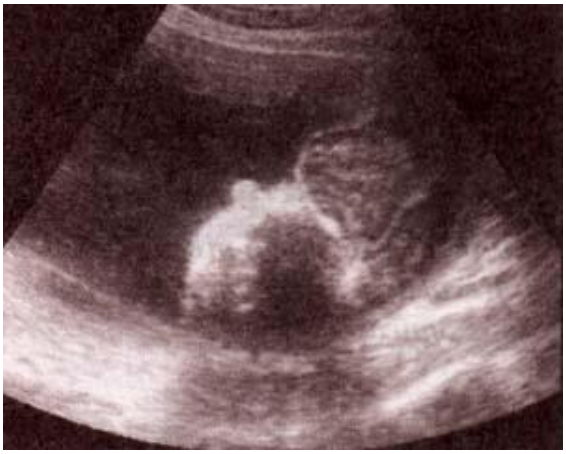
Anencephaly results from a failure to close the most anterior portion of the neural tube. Instead of the cerebral hemispheres, a mass formed by thin-walled vascular canals, known as the cerebrovascular area (angiomatous stroma), protrudes from the base of the skull. This vasculous brain mass appears to derive from the choroid plexuses and is covered by a continuous membrane.

Anencephaly was the first fetal malformation diagnosed by ultrasound in the prenatal period. The high resolution of the ultrasound machines currently used allows diagnosis in the first trimester. Golstein warns that, in the first trimester, the diagnosis should be made

with caution (especially before 11 weeks), due to the difficulty in differentiating the cerebrovascular area in early stages of pregnancy. With experienced sonographers, the diagnosis of anencephaly can be made in 100% of cases.

Prenatal diagnosis of anencephaly is based on the absence of a cranial vault. Between 12-13 weeks of gestational age it is totally abnormal not to observe bone structures that delimit the upper portion of the skull above the orbits. When the cerebrovascular mass is prominent, an ill-defined mass with a heterogeneous echostructure above the orbit is seen on ultrasound. Suspicion may arise when an attempt to measure BPD in the transaxial section in the thalamus cannot be located.





With the introduction of transvaginal ultrasound, early diagnosis of neural tube defects and other congenital anomalies has become more accessible and accurate (Bayle & Gregory, 1999).

One of the main challenges in the differential diagnosis of anencephaly is the sequence of amniotic bands, which can interfere with the normal formation of cranial structures. The key difference lies in the asymmetry of deformities caused by amniotic bands, in contrast to anencephaly, which is usually bilateral and symmetrical. In addition, amniotic bands are often associated with other abnormalities in the abdominal wall and extremities (166).

The prognosis for anencephaly is extremely poor, as most affected fetuses die in utero or in the first postnatal hours. However, the literature describes exceptional cases of neonates who have survived up to six months (209)

Approximately 33% of cases of anencephaly have associated congenital malformations, the most common being spina bifida. Other common abnormalities include malformations of the urinary system, cleft palate, and/or cleft lip, as well as congenital heart defects (163).

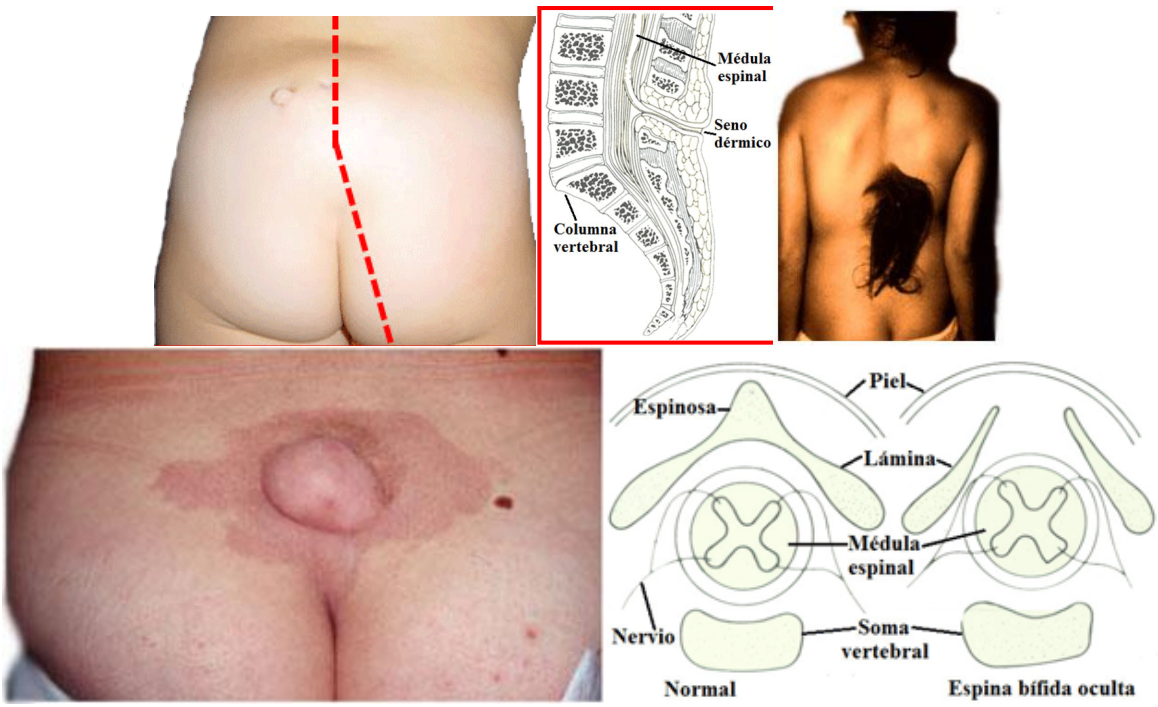
MYELOMENINGOCELE-SPINA BIFIDA

Myelomeningocele is the second neural tube closure defect in order of frequency, surpassed only by anencephaly. In comparison, isolated meningoceles are significantly rarer. This malformation originates from the lack of closure of the posterior neuropore between the third and fourth weeks of gestation, resulting in the exposure of the neural plate (154,157).

Neurological defects associated with myelomeningocele range in severity from mild anesthesia to complete paraparesis and even death. The prognosis is aggravated when the lesion is more extensive or more localized within the spine, as well as when it is associated with other congenital anomalies (210)

Ultrasound evaluation of the fetal spine is usually performed between 16 and 17 weeks of gestation. Under normal conditions, the distance between the posterior ossification centers is equal to the inter-pedunculated distance, which is measured between two consecutive vertebral pedicles. However, in spina bifida accompanied by myelomeningocele, an abnormal separation of the posterior ossification centers is observed on ultrasound examinations, both in transverse and longitudinal sections (166).

From the embryological point of view, it is postulated that a defect in primary neurulation—specifically in the closure of neural folds—can lead to malformations such as encephalocele, craniorachischisis, anencephaly, and cervicothoracic spina bifida (209). On the other hand, lumbosacral spina bifida seems to originate from a defect in spinal cord canalization, affecting secondary neurulation (163).



Spina bifida is a neural tube malformation that can occur in different clinical forms. It is classified into two large groups: spina bifida abierta and spina bifida occulta.

In open spina bifida, the spinal defect allows the externalization of nerve tissue, which results in the formation of a meningocele or myelomeningocele, depending on the involvement of the meninges and spinal cord. This type of presentation is frequently associated with severe neurological alterations and permanent disability.

On the other hand, spina bifida occulta is characterized by the presence of a bone defect covered by skin or by a thick opaque membrane, without externalization of neural tissue. In general, this variant affects the L5 or S1 levels and is usually asymptomatic or manifests with minor signs, such as a tuft of hair, lipomas or dermal pits in the lumbar region.

The terms "open" and "hidden" are determined by the presence or absence of meninges that protrude through the bone defect, regardless of their contents. Because more than 80% of the forms of spina bifida are myelomeningocele, the term "spina bifida" is often used to refer to this specific presentation.

Spina bifida mainly affects the lower portion of the spine, although there are rare forms of myelomeningocele at the thoracic, cervical, or upper levels. These variants usually have a more guarded prognosis and require a more detailed neurological evaluation.

To define the diagnosis and prognosis of spina bifida by ultrasound, the following aspects must be assessed:

1. Identification of the defect at the spinal level The evaluation of the dysraphic components can result in false positives and negatives, so it is essential to examine the area after the defect to identify the presence of a meningeal sac. Depending on their content, the following forms can be distinguished:

   Meningocele: Contains only cerebrospinal fluid.
   Myelomeningocele: It has nerve structures inside.
   The observation of a posterior sac is a key criterion for the diagnosis of spina bifida (166). Cystic forms or those containing nerve tissue are the most common. Cases of lipomeningocele with normal neurological evolution have been documented after removal of adipose tissue (Denis & Pedear, 1999).
2. Search for the effects of the defect on the central nervous system Among the associated abnormalities, the most frequent is ventriculomegaly, detected in approximately 75% of cases (Bayle & Gregory, 1999). Another common complication is Arnold-Chiari malformation, a disorder of the hindbrain that is a common cause of hydrocephalus. This malformation has two main components:

   to. Caudal displacement of the cerebellar vermis towards the upper cervical canal.

   b. Descent of the spinal cord and fourth ventricle (Wilkins-Haug, 1999).

   Classification of Arnold-Chiari malformation:

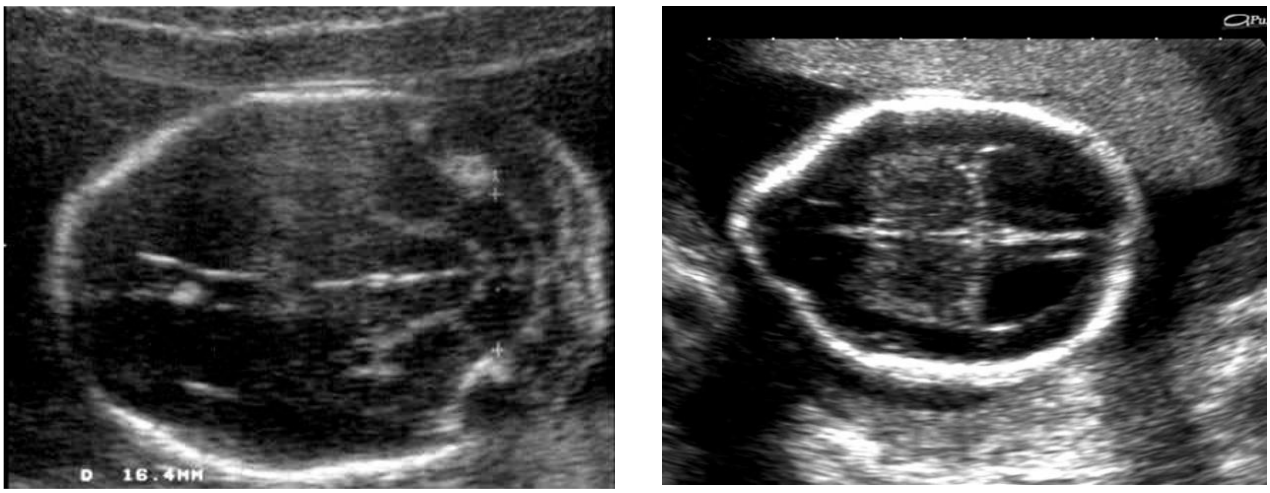
   c. Type I: Caudal displacement of the spinal cord.

   d. Type II: Descent of the spinal cord along with the fourth ventricle.

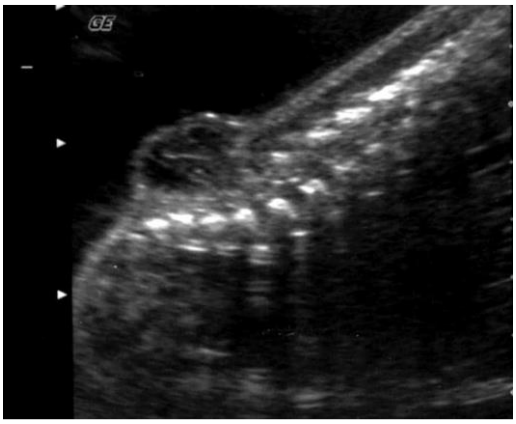
   and. Type III: Additional displacement of the cerebellum.

The most common form is type II, in which herniation of the cerebellum through the foramen magnum causes secondary compression of the Silvio aqueduct, resulting in hydrocephalus (Denis & Pedear, 1999).

Although Arnold-Chiari malformation is primarily associated with spina bifida, it can also occur along with other abnormalities such as polymicrogyria, hydrocephalus, subependymal heterotopia, Sylvian aqueduct stenosis, syringomyelia, and myelocystocele (166).



Cranial signs of myelomeningocele: Lemon sign (Left photo) and Banana sign (Right photo)



Lumbar spina bifida: Longitudinal section

In Arnold-Chiari malformation, in addition to ventriculomegaly, which occurs in 54-86% of cases, two characteristic ultrasound signs have been described:

1. Lemon sign: A deformation of the skull is observed in a cross-section, adopting a morphology similar to a lemon due to the retraction of the frontal bones. This sign usually disappears after 34 weeks of gestation (Wilkins-Haug, 1999).

2. Banana sign: It is caused by the displacement of the cerebellar hemispheres towards the cervical canal, which causes the obliteration of the cisterna magna (Bronshtein & Bar-Haval, 1992).

The impossibility of visualizing the cisterna magna is a finding present in more than 95% of cases of open spina bifida. In addition, normal visualization of the cerebellum has been reported to occur only in a maximum of 5% of affected cases. Other cranial signs associated with spina bifida include the presence of a biparietal diameter below the 5th percentile, with a frequency reported in 61% to 79% of cases (Denis & Pedear, 1999).

Myelocystocele is a lesion that has a close connection between a subcutaneous cystic structure and the central canal of the spinal cord. This cyst usually contains material

with characteristics similar to choroid plexuses (Carlson, 1994). Anatomically, cervical myelocystocele is made up of:

- A hydromyelinated sac.
- A meningocele covering.
- A spina bifida defect through which the lesion protrudes.

The prognosis of myelocystocele depends on the presence of associated cranial abnormalities, such as Arnold-Chiari malformation type II and hydrocephalus (Bayle & Gregory, 1999).

Differential diagnosis

The main conditions that should be considered in the differential diagnosis of myelocystocele include:

- Cystic hygroma.
- Cervical meningocele.
- Cervical myelomeningocele.
- Rare neoplasms, such as neuroblastic teratoma and hemangioma (Wilkins-Haug, 1999).

1. Assessment of the neurological consequences of the injury according to its extension, morphology and mobility of the lower limbs It is essential to evaluate neuromuscular disorders, ambulation capacity and control of urinary function. In severe cases, alterations such as clubfoot, congenital hip dislocation and paralysis of the lower limbs are observed, which leads to a reserved prognosis.

or In open forms, only 23% of patients achieve normal ambulation.

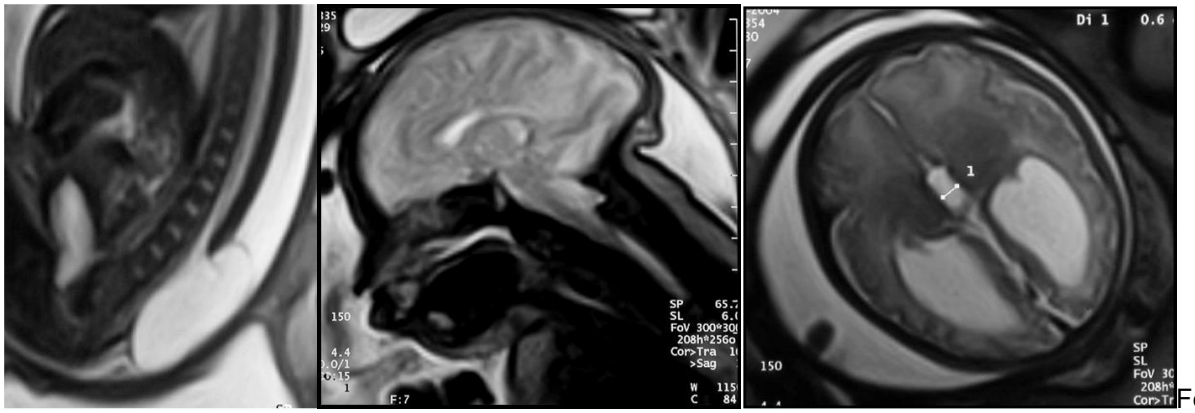
or 53% require orthopedic fixations on the lower extremities.

or The remaining 24% need the use of a wheelchair.

The sacral location of the lesion tends to have a better prognosis in terms of mobility, as only 17% of these patients require crutches or limb fixation, while the rest can walk without assistance. However, with regard to sphincter control, sacral lesions have the worst prognosis, with 100% of cases being affected.

3. Ruling out other abnormalities outside the central nervous system Spina bifida can be associated with multiple additional malformations, including:

1. Kyphoscoliosis.
2. Single umbilical artery.
3. Systemic malformations not related to the central nervous system.
4. Chromosomal diseases.


Fetal MRI: Left photo: Lumbar spina bifida. Medium photo: Arnold Chiari. Right photo: Ventriculomegaly

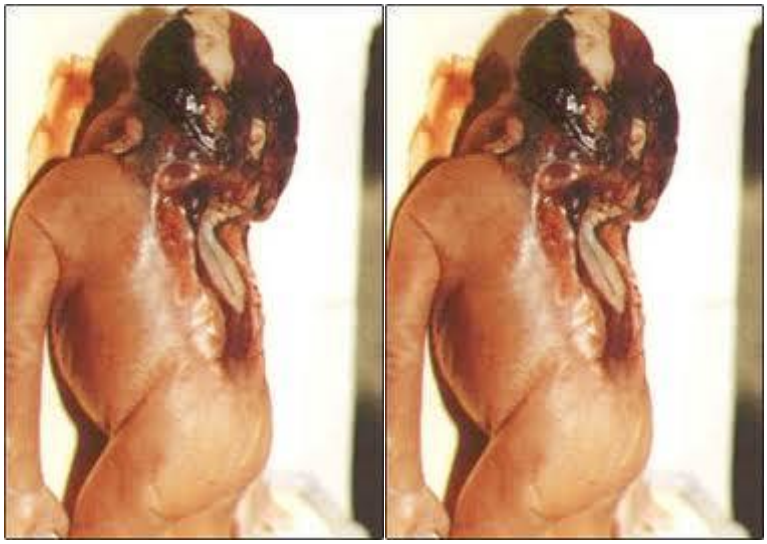


Fig. Fetus with severe rachitchisis. The brain is not covered by cranial bones, and the spinal cord is totally exposed. (Courtesy of Mario Pinos)

ENCEPHALOCELE

Encephalocele is defined as the protrusion of the meninges with or without brain content through a defect of the skull, resulting from an alteration in the process of closure of the upper pole of the neural tube. It is the least common entity among neural tube defects, with an estimated prevalence of approximately 1 case per 2000 live births (Carlson, 1994; Bayle & Gregory, 1999).

The etiological mechanism of encephalocele may involve an error in the separation process between the ectoderm and the neuroectoderm, leading to a mesoderm defect and, consequently, an alteration in bone formation. This allows the herniation of the meninges, either in isolation or with brain tissue inside them (Wilkins-Haug, 1999).

Location of the encephalocele

The most common sites of encephalocele presentation are:

- Occipital region: 75% of cases.

- Frontal region: 13% of cases.
- Parietal region: 12% of cases.

Ultrasound characteristics

The ultrasound manifestations of encephalocele vary depending on the size of the lesion, its location and its contents. Detailed ultrasound evaluation is essential for prenatal diagnosis and postnatal treatment planning (Bronshtein & Bar-Haval, 1992).

Encephalocele ultrasound patterns

Encephalocele ultrasound patterns can present in the following ways:

1. Cystic mass: Corresponds to a meningocele.
2. Solid mass: Contains structures recognizable as brain tissue.
3. Mixed pattern: Combination of cystic content and brain tissue.
4. Decreased head circumference: In 20% of encephalocele cases, displacement of the brain mass causes microcephaly.

In posteriorly located masses, the differential diagnosis should be established with cystic hygroma, which is distinguished by the absence of a bone defect, the continuity of the skin and surrounding subcutaneous cellular tissue, as well as the presence of internal septa in the hygroma (Carlson, 1994).

Differential diagnosis and associated syndromes

Lesions located outside the midline are usually associated with amniotic flange syndrome, although antenatal AFP screening programs have improved early identification of cephaloceles. Most of these are covered with skin and, therefore, do not have an increase in AFP levels (Bayle & Gregory, 1999).

Some extracranial masses detected during the prenatal period that require differential diagnosis include:

- Cystic hygroma.
- Soft tissue edema.

- Mesenchymal sarcoma.
- Occipital hemangioma, which may mimic an encephalocele (Bronshtein & Bar-Haval, 1992).

Several genetic syndromes have been associated with encephalocele, including:

- Von Voss syndrome.
- Knoblock syndrome.
- Chemke syndrome.
- Disegmentary dysplasia.
- Cryptophthalos syndrome.
- Roberts syndrome.
- Walker-Warburg syndrome.
- Amniotic band syndrome.
- Agenesis of the corpus callosum.
- Meckel syndrome.
- Dandy-Walker syndrome (Carlson, 1994; Bayle & Gregory, 1999).

Nasal teratoma should be distinguished from frontal encephalocele, as teratomas have an irregular mass with a more heterogeneous architecture. Likewise, the deformation of the skull into a "clover leaf" can simulate the appearance of an encephalocele (Bronshtein & Bar-Haval, 1992). Dacryocystoceles (cysts of the tear duct) can also be confused with encephaloceles (Carlson, 1994).

Prognosis

The prognosis of encephalocele depends on several factors, including:

- The presence of brain tissue in the herniated meningeal sac.
- Associated malformations, whether syndromic or not.
- The presence of hydrocephalus and/or microcephaly.
- The location of the encephalocele: the anterior ones have a better prognosis in terms of survival compared to the posterior ones.

The survival rate varies between 80% and 93%, depending on the factors mentioned (Bayle & Gregory, 1999).

GENETIC ENHANCEMENT

Until a few years ago, the only means of preventing neural tube closure defects (NTD) was genetic counseling, with the aim of preventing future pregnancies in couples with a history of congenital malformations. Many families at high risk of recurrence chose to limit the size of their offspring, as the likelihood of recurrence directly influenced their reproductive decision.

For example, couples with one or two children affected by DCTN faced recurrence risks of 1:20 or 1:8, respectively, leading them to avoid future pregnancies. However, when the risk of recurrence was reduced to 1:100, couples tended to accept new pregnancies if they wanted to have more children.

The risk of recurrence after an affected pregnancy is approximately 5% (1:20), but this value may increase if the parents come from regions with a high prevalence of TNCD. In contrast, in populations with a low prevalence of the pathology, the risk of recurrence is lower. Assessment of the risk of recurrence is a crucial factor in family planning and reproductive decision-making (Carlson, 1994; Bayle & Gregory, 1999). (Table 5).

TABLE 5 Risk of DCTN Using in Genetic Counseling

Risk situation	Relative risk
General population	1:200
A previous affected child	1:20
2 previous affected children	1:10
An affected parent	1:25
An affected uncle or aunt	1:50
A cousin or great-uncle or uncle	1:100
Other related	1:200

Until a few years ago, the only means of preventing neural tube closure defects (NTD) was genetic counseling, with the aim of preventing future pregnancies in couples with a history of congenital malformations. Many families at high risk of recurrence chose to limit the size of their offspring, as the likelihood of recurrence directly influenced their reproductive decision.

For example, couples with one or two children affected by DCTN faced recurrence risks of 1:20 or 1:8, respectively, leading them to avoid future pregnancies. However, when the risk of recurrence was reduced to 1:100, couples tended to accept new pregnancies if they wanted to have more children.

The risk of recurrence after an affected pregnancy is approximately 5% (1:20), but this value may increase if the parents come from regions with a high prevalence of TNCD. In contrast, in populations with a low prevalence of the pathology, the risk of recurrence is lower. Assessment of the risk of recurrence is a crucial factor in family planning and reproductive decision-making (Carlson, 1994; Bayle & Gregory, 1999).

The risk increases in families from disadvantaged social classes, especially when the mother receives an insufficient diet or there is a family history of the condition. Similar risks have been identified in cases where one or both parents have complicated cystic or occult spina bifida. Although men with motor disabilities and lower limb paralysis may have offspring, women with spina bifida often have complications during childbirth.

If a second-degree relative (sibling of a parent) is affected, the risk of recurrence approaches 1:50. This risk increases if more family members are affected. In the case of a third-degree relative, the risk decreases to less than 1:100, approaching the general prevalence of the population. If a couple has a child with DCTN and conceives with a new partner, the risk of recurrence is reduced from 1:20 to 1:30.

The risk is equivalent regardless of whether the first case was anencephaly or spina bifida, and does not vary between men and women. Nonetheless, it is essential to warn couples with a history of DCTN about the likelihood that their offspring will also be affected.

The risk of recurrence depends on the prevalence in the population. In regions such as South Wales, a reduction in the risk of recurrence has been documented in the last decade, attributed to better access to genetic counselling and antenatal diagnosis.

When TNCDs are part of a malformation syndrome, the risk of recurrence is adjusted according to the underlying pathology. For example, in autosomal recessive Meckel-Gruber syndrome, the risk for siblings is 25% (1:4).

Prenatal diagnosis of DCTN has been incorporated into genetic counseling, complemented by dietary strategies and folic acid or multivitamin supplementation. However, multicenter studies by the Medical Research Council have pointed out that multivitamin supplements contain insufficient amounts of folic acid, which has led to debate about their preventive effectiveness. Women at risk are recommended to use oral contraceptives during the first two months of supplementation to avoid pregnancy during that period.

In addition, women with maternal fever above 38.9°C during the first trimester of pregnancy have been identified as having an increased risk of DCTN, requiring dietary counseling and specialized follow-up (Chamber et al., 1998).

PRENATAL DIAGNOSIS OF DCTN IN HIGH-RISK PREGNANCIES

Currently, most neural tube closure defects (NTCDs) can be identified by prenatal tests performed during the second trimester of pregnancy. Prenatal diagnosis in this period includes high-resolution ultrasound and amniocentesis, which allows measurement of alpha-fetoprotein (AFP) and other investigations in the amniotic fluid.

None of these diagnostic procedures should be performed without prior genetic counseling to the parents, in which they are informed about the purposes, risks and implications of the tests. This counseling is crucial for informed decision-making and for understanding the impact of the diagnosis on family planning.

It is essential to provide psychological and emotional support during the diagnostic process and in the waiting period for results, since this period generates anxiety and tension in parents. Uncertainty about fetal health status and the possibility of facing difficult decisions require a multidisciplinary approach in the management of high-risk pregnancies.

The combined use of advanced prenatal imaging techniques and serum biomarkers improves the accuracy of diagnosis and assesses the severity of DCTN, facilitating a timely medical and therapeutic approach.

HIGH-RESOLUTION ULTRASOUND

Detailed ultrasonography is performed when there is an elevated risk of fetal abnormalities, especially in women with a family or obstetric history of neural tube closure defects (NTCD), elevated maternal serum alpha-fetoprotein (AFP) levels, and suspicious ultrasound findings of fetal abnormalities prior to or prior to amniocentesis. Its use as a generalized screening procedure is not recommended (Wilkins-Haug, 1999).

With advances in real-time, high-resolution ultrasound, an experienced specialist can accurately detect both major defects, such as anencephaly and severe spina bifida, as well as smaller lesions. This procedure, being non-invasive and safe, has not demonstrated adverse effects on the mother or fetus at the intensities and frequencies used in diagnosis (Bronshtein & Bar-Haval, 1992).

Anencephaly can be identified by ultrasonography as early as 11 weeks' gestation, while spina bifida can be seen as early as 14 weeks, but a more detailed evaluation is recommended at 16 weeks, when abnormalities are most evident (Denis & Pedear, 1999).

On the longitudinal axis, the normal fetal spine is visualized as two continuous parallel lines converging in the sacral region and diverging in the upper cervical region. A spinal defect, such as spina bifida, causes loss of parallelism, expansion, and irregularities in the morphology of the spine (Christensen et al., 1999). In the cross-section, instead of the normal bony ring, an open "U" or "V" shaped canal is detected. In some cases, cranial deformation with the "lemon" sign and the presence of severe hydrocephalus may be additional indicators of spina bifida (Acuna & Yoom, 1999).

Other abnormalities that may be detected include widening of the cervical spinal canal, renal malformations (polycystic disease, renal agenesis), and foot abnormalities

(Blumfeld et al., 1993). The absence of movement in the lower extremities is not a conclusive sign of spina bifida. However, ultrasound evaluation may be limited in cases of oligohydramnios or when open spina bifida presents a large meningocele, which can be confused with other masses such as lipomas or sacrococcygeal teratomas, entities with a better prognosis (164).

Encephalocele is a congenital anomaly that presents significant difficulties in its identification by prenatal ultrasound. Its differential diagnosis is complex, since it can be confused with other pathologies such as nuchal cyst, which is associated with various chromosomal abnormalities and may present a similar ultrasound picture (166).

Since encephaloceles are closed lesions, their biochemistry in the amniotic fluid is normal, unlike other open neural tube defects such as spina bifida and anencephaly, in which a significant increase in alpha-fetoprotein (AFP) in the amniotic fluid is observed (Denis & Pedear, 1999). Large encephalocele lesions are usually associated with microcephaly, while smaller ones may present with normal cephalic size, making prenatal detection difficult (162).

When spina bifida or encephalocele is identified by ultrasound, an amniocentesis is recommended for biochemical analysis of the amniotic fluid, in order to confirm the diagnosis and avoid diagnostic errors (148). In addition, it is important to perform cell cultures and cytogenetic studies, since in some cases the presence of an encephalocele may be associated with specific chromosomal abnormalities, such as trisomy 13 or trisomy 18 (211).

In contrast, amniocentesis does not play a relevant role in the diagnosis of anencephaly, since this malformation is easily identifiable by ultrasound due to the absence of the cranial vault and the presence of exposed neuroectodermal tissue (164).

BIOCHEMISTRY OF AMNIOTIC FLUID

In 1972, Brock et al. in Edinburgh discovered that pregnancies with an open lesion, such as anencephaly and open myelocele, are associated with increased levels of alpha-fetoprotein (AFP) in the amniotic fluid at the beginning of the second trimester (212). AFP is a glycoprotein produced primarily by the fetal liver, reaching its peak concentration between 17 and 18 weeks of gestation (211). Normally, AFP remains in the fetus and only small amounts pass through the fetal kidney into the amniotic fluid, where its concentration can be measured by immunoelectrophoresis, radioimmunoassay, immunoradiometric or enzyme-linked immunosorbent assay (162).

The upper limit of normal AFP in amniotic fluid remains stable until 13 weeks of gestation, peaking at about 40 Ku/mL at 15 weeks, and then decreasing to variable levels below 20 Ku/mL by 21 weeks (Denis & Pedear, 1999). In the presence of an open lesion, such as anencephaly with angiode neural tissue at the base of the skull or an open myelocele with the neural plate exposed, AFP leaks into the amniotic fluid, resulting in considerably elevated levels: approximately 60 Ku/mL in spina bifida and 100 Ku/mL in anencephaly (Wilkins-Haug, 1999).

AFP values can be expressed in absolute values or as multiples of the median (MoM) for a given gestational age. An AFP value in amniotic fluid above 3 MoM is considered abnormal and highly indicative of a neural tube closure defect (148). However, in skin-covered lesions, such as some closed anencephalies or encephaloceles, AFP levels in the amniotic fluid remain within normal ranges (166). Spina bifida occulta is also not associated with elevations in AFP and usually does not present with concomitant hydrocephalus (164).

In addition to neural tube defects, elevated levels of AFP in the amniotic fluid may be seen in anterior abdominal wall defects, fetal death, and congenital nephrosis. They can also be found in increased renal malformations, urethral obstructions, intestinal abnormalities, and genetic syndromes such as Turner syndrome (211). However, there are false positives, with an incidence of approximately 1 in 500 amniocentesis, which could lead to the erroneous termination of a normal pregnancy. In some cases, elevated AFP levels obtained after week 19 have led to misdiagnoses, underscoring the importance of comprehensive case evaluation by a multidisciplinary team that includes obstetricians, geneticists, and radiologists (162).

The acetylcholinesterase isoenzyme has been shown to be observed exclusively in open neural tube closure defects (NTCDs), allowing its detection by polyacrylamide gel electrophoresis as a specific second band (Denis & Pedear, 1999).

Electrophoresis shows two main bands:

1. Upper band: Represents the pseudo-cholinesterase present in the amniotic fluid.
2. Lower band: Corresponds to acetylcholinesterase derived from the extracellular space in the central nervous system or ganglion (211).

The lower band is inhibited with low concentrations of physostigmine olisivane, while the upper band shows no inhibition. In the presence of open DCTN, the lower band is reduced or absent, which allows differentiating these defects from other congenital pathologies.

It has been observed that the lower band may be attenuated in certain defects of the anterior abdominal wall and occasionally in other malformations. In cases of contamination with fetal (but not maternal) blood, a wide band is detected, which is inhibited by physostigmine, allowing the differentiation of false positives in the alpha-fetoprotein (AFP) test (162).

The combined use of AFP measurement and acetylcholinesterase detection has significantly improved the diagnostic accuracy of open DCTNs, reducing the rate of false positives in procedures such as amniocentesis. In South Wales, the routine implementation of these tests since 1981 has prevented erroneous decision-making in the termination of pregnancy (148).

Nine out of ten DCTNs can be identified by elevated levels of AFP in amniotic fluid, but the combination with the detection of acetylcholinesterase allows greater diagnostic certainty. For couples with a history of DCTN, this technique has reduced the risk of recurrence from 1:20 to less than 1:200, significantly improving acceptance of prenatal testing and informed decision-making about pregnancy continuity (164).

Amniocentesis remains the standard procedure for prenatal diagnosis of TNCD and is recommended between 15 and 18 weeks of gestation. Before 15 weeks, fetal risk is elevated, while after 19 weeks, the interpretation of AFP values becomes complicated, increasing the probability of diagnostic errors (Maestri et al., 1999).

Before proceeding with an amniocentesis, it is recommended to perform an ultrasound to: A. Confirm gestational age, since AFP values depend on the time of gestation. B. Locate the placenta to determine the appropriate amniocentesis technique. C. Exclude twin pregnancies, thus avoiding unnecessary or inappropriate procedures. D. Identify severe fetal abnormalities, such as anencephaly or stillbirth (Denis & Pedear, 1999).

Couples should be counseled about the scope and limitations of diagnostic testing. A normal acetylcholinesterase and AFP result does not guarantee the absence of closed lesions or other structural abnormalities. Likewise, amniocentesis carries a risk of miscarriage of less than 0.5%, but it must be performed by experienced professionals to minimize complications (212)

Maestri et al. (1999) established normal AFP values in amniotic fluid between 14 and 21 weeks of gestation, providing an essential reference for the interpretation of the results:

- 14 weeks: 16.32 KUI/ml
- 15 weeks: 14.36 KUI/ml
- 16 weeks: 10.93 KUI/ml
- 18 weeks: 8.22 KUI/ml

- 19 weeks: 7.35 KUI/ml
- 20 weeks: 5.62 KUI/ml
- 21 weeks: 4.47 KUI/ml

These values, together with the detection of acetylcholinesterase, have optimized the prenatal diagnosis of DCTN and have allowed more informed decisions to be made in high-risk pregnancies.

Prenatal diagnosis will be offered to women who have had a child with neural tube closure defects (NTCD) or in parents who have spina bifida. Amniocentesis will be performed if the risk of DCTN is 1:100 or less. This guides the couple on the continuity of the pregnancy or reduces parental anxiety. When the risk is low, a high-resolution ultrasound and alpha-fetoprotein (AFP) dosing will be offered between 16 and 17 weeks of gestation in maternal serum. Amniocentesis will be done if research indicates an increased risk. This strategy has the advantage of avoiding unnecessary risks to pregnancy, although one disadvantage is that one-fifth of DCTNs are associated with normal serum AFP levels.

Women who had amniocentesis in the second trimester should be monitored until the term of pregnancy to identify possible previously undiagnosed fetal abnormalities. Any spontaneously aborted fetus will be sent to pathology to determine the primary cause and extent of the malformation.

The impact of prenatal diagnosis on reproductive behavior was investigated in two groups of non-pregnant women who sought genetic counseling for DCTN. One group accessed counselling prior to the introduction of antenatal diagnosis, while the other was offered antenatal diagnosis. Both groups were followed for three years. Reproductive behavior was similar in both, with the same proportion of couples choosing not to have any more pregnancies. One of the effects of prenatal diagnosis was that it allowed high-risk couples to make faster decisions about future pregnancies. However, the test did not reveal all severe cases of spina bifida. 90% of the women in the second group who became pregnant opted for prenatal diagnosis. These women did not accept terminating the pregnancy or assuming the risk of miscarriage after amniocentesis. Almost all received counselling, but the time between amniocentesis and AFP results was a period of great distress.

Prior to the implementation of prenatal diagnosis for DCTN, women reported anxiety throughout pregnancy. Since the introduction of the test, a decrease in anxiety has been reported, and some women experience a reduction in the joy of pregnancy until AFP results are obtained.

It is emphasized that the interruption in the second trimester of a desired pregnancy is a very traumatic experience for both parents, very different from that of an unexpected stillbirth or neonatal death. These mothers usually receive little emotional help. Grief resolution and post-abortion support at genetic clinics or with genetic counselors are essential for emotional recovery and planning for future pregnancies.

PREGNANCY SCREENING FOR DCTN

Nine out of ten DCTNs are born to couples with irrelevant family or obstetric histories. Screening all pregnant women is a non-invasive, low-cost procedure that can be applied in populations with a high prevalence of DCTN.

Ultrasound screening is performed in the antenatal clinic at 16 weeks gestation and is a routine procedure in the United Kingdom. Its objective is to confirm gestational age by biparietal measurement and exclude multiple pregnancies. Although some fetal abnormalities such as anencephaly can be identified, a complete screening for malformations requires time, patience, operator skill, and sophisticated equipment. A fetal abnormality suspected by ultrasound must be confirmed with high-resolution fetal ultrasound and amniocentesis before decisions are made about termination of pregnancy (United Kingdom Collaborative Study, 1977).

The most common non-invasive screening is maternal serum **alpha-fetoprotein (AFP)** determination . Under normal conditions, AFP is not found in the maternal circulation; however, at 12 weeks gestation, small amounts cross the placental barrier. Its concentration increases until it peaks between 25 and 29 weeks, and then gradually decreases until birth. Between 16 and 19 weeks of gestation, maternal serum AFP is usually below **120 Ku/L** and is measured by radioimmunoassay or other similar methods. As in amniotic fluid, serum AFP levels are expressed in **multiples of the median (MoM)** for gestational age, with any value above **2.5 MoM considered abnormal** (López-Miranda et al., 2001).

Open lesions such as anencephaly and spina bifida are often associated with elevated AFP levels, although some fetal abnormalities may present normal values. An increase in serum AFP at week 16-19 may be due to small fetal-maternal bleeding, threatened miscarriage, missed abortion, twin pregnancy, or an open DCTN. This is due to a physiological quirk that allows more AFP to cross the placental barrier. In the event of an elevated serum AFP level, high-resolution ultrasound, amniocentesis for the determination of AFP in amniotic fluid, and the detection of **acetylcholinesterase** by gel electrophoresis

are recommended to identify the cause of the increase before considering termination of pregnancy (212)

A collaborative study in the United Kingdom evaluated the efficacy of screening for DCTN by measuring AFP in maternal serum. It was concluded that approximately **80% of open lesions** can be identified by elevated AFP levels between 16 and 19 weeks of pregnancy. Before week 16, detected cases may be unsuccessful, while after week 19, the usefulness of screening decreases due to variability in AFP levels, which can lead to late amniocentesis and late-stage termination of pregnancy (UK Collaborative Study, 1977).

López-Miranda et al. (2001) conducted a screening study for AFP in maternal serum in **1,336 pregnant women** between **16 and 18 weeks of gestation**, finding that in **30 pregnancies** AFP was elevated. Of these, **three** were associated with DCTN, while the others were false positives, related to omphalocele (2 cases), oligohydramnios (1 case), prematurity (1 case), fetal losses (3 cases), placental anomalies (4 cases), chorionic biopsy (2 cases), twin pregnancies (8 cases), and **6 cases without fetal or neonatal anomalies**. In addition, this same group of researchers reported a case of **elevated maternal serum AFP** associated with **fetal akinesia-hypokinesia syndrome type I** (López-Miranda et al., 2001).

A screening programme carried out in **South Wales**, with monitoring for **2.5 years**, identified organisational problems and difficulties in the population. The success of the AFP screening program was determined to depend on several factors, including the involvement of a reporting professional committee (obstetricians, nurses, and associated staff), integration between prenatal clinics with small-gestation ultrasound, an AFP laboratory, a referral center for high-resolution ultrasound, amniocentesis, and genetic counseling (Maestri et al., 2003).

Mothers are informed about early *pregnancy screening*, so they can decide if this approach to DCTN prevention is acceptable for them. In practice, many women are not informed, needing to be seen in antenatal clinics between **16-18 weeks** of pregnancy. The blood sample for the determination of **alpha-fetoprotein (AFP)** must be transported to a supraregional laboratory, using automatic techniques with strict quality control. The result will be available at the antenatal clinic within **seven days** (Jones et al., 2010).

Obstetricians interpret the results based on gestational age and ultrasound recorded at the antenatal clinic to provide an explanation to parents. In the event of an increase in AFP levels, mothers are referred to a specialized center for counseling, further investigations, and possible amniocentesis to identify the causes of abnormal AFP levels. (213)

Table 6: 1 of 9 women with an increased AFP level has a fetus with an open neural tube defect. Many parents of these pregnancies believe that the tests may be wrong due to their elevated serum AFP level, leading to bewilderment and resistance to further research (Smith et al., 2015). It is critical to provide support during this process, especially in waiting for results and making decisions about continuing the pregnancy in the event of a severe fetal abnormality (Brown & Thompson, 2018).

In a study conducted to evaluate AFP in maternal serum, considering effects of special provision, geographic location, and patient safety status in **Washington State**, it was shown that **80.4%** of urban areas and **77.0%** of rural areas obtained information through obstetrics and gynecology services. In addition, **64.2%** of urban areas and **62.2%** of rural areas received information from obstetricians and family doctors. In terms of insurance coverage, **Medicaid** was less representative at **60.5%,** compared to **79.1%** for private insurers. These data reflect efforts to ensure that all patients receive adequate information about *screening* for DCTN (Johnson et al., 2017).

The cost of performing serum AFP is low; no AFP screening program is expensive or inaccessible. However, in regions of low prevalence, many obstetricians prefer not to inform mothers about this option, despite the possibility of detecting significant fetal abnormalities (214)

A study conducted on **2803 pregnant women** using **triple *screening*** (*maternal serum alpha-fetoprotein, chorionic gonadotropin beta subunit, and unconjugated estriol*), showed that **95%** of cases were associated with fetal abnormalities in general. In addition, **87%** were related to **Down syndrome** and **85.5%** to **neural tube closure defects** (Garcia et al., 2019).

Table 7: In summary, the implementation of maternal serum AFP screening for DCTN is presented in the corresponding table (213)

TABLE 6 **Causes of increased levels of AFP in maternal serum and associated malformation.**

On estimation of gestational age Multiple pregnancy Loss of AFP from the fetus through abnormal tissues Increased fetal excretion AFP Increased AFP concentration in aniotic fluid	DCTN (anencephaly, myelomeningocele) Abdominal wall defects (omphalocele, gastrochisis) Nuchal membrane (frequently to aneuploidy) Stillbirth Fetal teratoma Decreased renal resorption (congenital nephrosis, polycystic kidney) Oligohydramnios (agenesis, urethral obstruction) Decreased fetal swallowing (duodenal or esophageal atresia)

TABLE 7 **Maternal serum screening for DCTN and Down syndrome**

Factor	DCTN	Down syndrome
Time	16-18 weeks	16-18 weeks
Analyzed Marker	AFP	AFP hCG, uE3
Percentage of women who choose the screening test	80-90%	70-80
Percentage of women at high risk	3-4%	4-5
Ultimate Test	Ultrasound with amniocentesis in some cases	Fetal karyotype
Final result time	Immediate	2-3 weeks
Percentage of high-risk women who have the definitive test	100%	70-80
Percentages of high-risk women who have affected fetuses	2-3%	2-3
Percentage of affected fetuses detected	70-80%	50-60

Prenatal diagnosis for DCTN was introduced in South Wales between 1973 and 1987 over 5000 amniocentesis indicated for DCTN (Table 8) leading to the identification and termination of 297 affected pregnancies.

Table 8 **Amniocentesis indication for DCTN in South Wales 1974-1987**

DCTN DETECTADOS					
Indicaciones	Numeros	Anencefalia	Espina Bifida Quistica	Encefalocele	Relacion
DTN previo	1611	17	16		1:49
Historia familiar de DT	1299	5	2		1;186
Padre con un DTN	69	-	-		-
AFP materna serica alta	2136	81	144	5	1;9,3
Ecografia anormal	65	22	15		1;1,8
Total	5120	177	177	5	

Prenatal diagnosis in women who had a previous **neural tube defect (NTCD)** is a common practice, although affected pregnancies are relatively few. The low recurrence rate (**1:49**) is due to the implementation of dietary advice and improved maternal feeding. Since 1981, a significant proportion of women have received preconceptional supplementation with **folic acid** or *Pregnative Forte*, which has contributed to the reduction of DCTN. (215)

The increase in early detection of DCTN is also associated with the implementation of **AFP screening programs**, introduced in **1976**, which has allowed the early identification of DCTN and has contributed to the decrease in **spina bifida** cases in some regions of the United Kingdom (Smith et al., 2002). Thus, **amniocentesis** was performed in women with a family history of TNCD in a ratio of **1:200**, although this practice has decreased with the use **of high-resolution ultrasound** and serum AFP screening (Jones et al., 2005).

Since the introduction of **high-resolution ultrasound**, amniocentesis is performed to confirm the presence of DCTN before considering termination of pregnancy. (216)

Dawson et al. conducted a study in which they analyzed the levels of **folate and vitamin B12** in amniotic fluid in pregnant patients with a history of DCTN. These levels were found to be **below the normal range** compared to pregnancies without DCTN, suggesting a possible relationship between the metabolism of these micronutrients and the occurrence of DCTN. (215)

A recent trend has been termination **of pregnancy based solely on ultrasound findings**, without a second ultrasound evaluation or confirmation by amniocentesis. While this may be acceptable in cases of **anencephaly**, it is considered **unwise** for other DCTNs, as it could lead to the termination of pregnancies of normal fetuses or those with minor abnormalities. (217)

The reduction in the number of births with DCTN is largely due to the **termination of affected pregnancies**, which has resulted in a decrease in the frequency of DCTN since the mid-1970s in the UK. This resembles the decline seen in **North America in the 1950s** (218) . However, this decline can also be attributed to **improvements in living standards**, **maternal nutrition**, and changes in demographic patterns, as social classes at higher risk tend to have fewer children. (219)

PRIMARY PREVENTION

Poor maternal nutrition is a determining factor in the genesis of neural tube closure defects (NTCD). The beneficial effect of dietary advice and improvement was investigated in South Wales between 1969 and 1975. The inclusion of foods rich in folic acid (FA), particularly meat, eggs, milk, plenty of lightly cooked fresh green vegetables and fruits, was recommended. In addition, it was suggested to replace refined white bread with whole wheat bread and avoid canned preserves as much as possible, as well as foods with a high content of dietary fiber. It was also recommended to reduce the excessive consumption of starch, refined carbohydrates, pastries, fats and sweet alcoholic beverages

Dietary advice has been shown to be effective; approximately **75%** of mothers significantly improved their diets in subsequent pregnancies. In (220) **109 prospective pregnancy studies** with dietary counseling, the risk of recurrence was halved than expected; in contrast, **77 unadvised pregnancies** maintained the predicted risk of recurrence. The difference was significant: (221) **3 cases of recurrence among the counseled women** and **5 cases in the non-counseled group**, highlighting the importance

of nutrition education and counseling in the prevention of DCTN (Table 9) (*Wald et al.*, 2001).

Table 9 **DCTN Dietary Study of Projected Pregnancies (Number of Pregnancies)**

	Calidad de dieta								
		Asesoramiento			No asesoramiento				
	Bueno	Regular	Pobre	Total	Bueno	Regula	Pobre	Total	Total
Resultado Norma	40	46	10	96	13	39	12	64	160
Recurrencias	-	-	3	3	-	-	5	5	8
Abortos	-	3	3	7	-	-	8	8	18
Total	40	49	20	20	39	25	77	77	186

At the same time as the dietary study in South Wales, a double-code-blind placebo-control trial was conducted; giving 4 mg of folic acid per day. No recurrence was found in 44 women who were supplemented; while there was recurrence in 63 women who took placebo and 16 who did not supplement folic acid (Table 10).

Table 10 **Results of folic acid supplementation in a group of treated pregnant women**

Folate Supplement				
	Meet	They do not comply	Placebo	All
Normal fetus	44	14	59	117
DCTN	0	2	4	6
All Results	44	16	63	123

Similar results were reported in Cuba, in a small study (28). This is a pharmacological dose that outweighs not only the low folate diet, but maternal metabolic problems and absorption problems. Smithells et al. found that folic acid and some vitamins were lower in women of social classes IV and V than in others during the first trimester of pregnancy; a periconceptional multivitamin, containing 12 substances including vitamins A, B, C, D, and E and some folic acid (pregnative Forte F) reduced recurrence (Table 11).

Table 11 Mothers supplemented and not supplemented with Pregnative Forte F

	Supplemented	Not supplemented
Number of mothers	454	529
Normal Result	429	570
Abortions Examined	30	19
Recurrence	3 (0.7%)	24 (4.7%)

Since 1982, supplementation with 5 mg per day of folic acid has been used in South Wales in 172 women, all of whom had one affected child; with 2 recurrences, the result is similar to that obtained by Smithells et al. (Table 12)

These studies had major methodological problems. In the study of multivitamin supplementation, the small and high nonconformity index. In this approach prevention is important, the Medical Research Council in the United Kingdom, launched an international placebo-control multicenter study of 4 mg folic acid and multivitamin vs multivitamins and folic acid. This study, which was carried out with some ethical problems when using placebo, does not yet produce conclusive results.

Table 12 Folic Acid Supplementation since 1982

Results	
Normal result	153
Abortions	12
Recurrence (1 spina bifida, 1 encephalocele)	2
Other malformations (1 klinefelter, 1 CDH, 2 bot foot, 1 pylorus stenosis)	5
Total supplemented	172

NUTRITIONAL AND PHARMACOLOGICAL PREVENTION OF NEURAL TUBE CLOSURE DEFECTS (NCD)

Pending the results of the *Medical Research Council (MRC)* study, it is recommended that mothers at increased risk of DCTN receive dietary counseling. These recommendations include preventing pregnancy within the first two months by using oral contraceptives and restricting the use of unnecessary medications, such as anticonvulsants. (221)

Supplementation with **5 mg of folic acid per day** or use of *Pregnative Forte F.* before conception and until the end of the first trimester contribute to reducing the risk of DCTN. This protocol has been widely accepted in the medical community as an effective strategy for the primary prevention of these defects. (220)

LIPOIC ACID AND ITS ROLE IN THE PREVENTION OF DIABETIC EMBRYOPATHY

Wiznitzer et al. have evaluated the impact of **lipoic acid** as a preventive alternative in diabetic embryopathy. Their findings suggest that this compound could significantly reduce the incidence of neural tube defects associated with maternal diabetes. A protective effect of lipoic acid against diabetic embryopathy, fetal loss, and ultrastructural alterations of the diabetic placenta has been evidenced (222).

IMPORTANCE OF INOSITOL IN NEURAL DEVELOPMENT

Recent advances in nutritional and biochemical research have identified the relevance of **inositol** in cell metabolism. Its potential in the prevention of DCTN in animal

models, particularly in mouse embryos, has been evaluated (223). An in vitro study conducted on myometrial tissue from non-pregnant rats showed that myo-inositol can significantly increase uterine contractility. This effect is attributed to the regulation of intracellular calcium fluxes, a mechanism similar to that of oxytocin, a key hormone in the induction of labor. The results showed increases in the frequency and amplitude of contractions with increasing doses of myo-inositol, suggesting a dose-dependent effect. (224). In contrast to findings in animal models, human clinical studies have indicated that myo-inositol supplementation during pregnancy is safe and not associated with increased uterine contractions. For example, a pilot study conducted in women with a history of pregnancies affected by neural tube defects (NTD) found that administration of myo-inositol along with folic acid did not cause side effects or severe uterine contractions, (225).

SCIENTIFIC EVIDENCE

SCIENTIFIC EVIDENCE ON THE PREVENTION OF NEURAL TUBE CLOSURE DEFECTS (NCD)

In 1964, Hibbard reported an association between birth defects and folate deficiency. Subsequently, in 1976, Smithels et al. established a link between the deficiency of folate and some vitamins with the recurrence of DCTNs. In 1980, the results of multivitamin supplementation were published, showing that the recurrence of DCTN was 5% among women who did not take supplementation, while in those who did it was only 0.6%. (226) (227) (220)

Laurence (1980) suggested that women with an adequate diet would have a lower risk of DCTN recurrence. In 1981, he published the results of a trial that demonstrated a 60% reduction in the risk of DCTN recurrence, although not statistically significant). During the 1980s, four observational studies were published, all of which showed that the administration of folic acid and multivitamins during the periconceptional period had a protective effect against DCTNs. (228) (221)

In the early 1990s, two observational studies and one nonrandomized clinical trial reinforced these findings, demonstrating that maternal supplementation with folate and folic acid significantly reduced the incidence of DCTN. (229)

In 1991, the **Centers for Disease Control and Prevention (CDC)** published a review of the scientific evidence for preventing recurrence of pregnancies affected by DCTN (*CDC, 1991*). Based on these findings, the **U.S. Public Health Service** recommended that all women of childbearing age consume **0.4 mg of folic acid per day** to reduce the risk of DCTN (230)

In 1992, official recommendations were issued in the United States, stating that all women of reproductive age should consume 0.4 mg of folic acid daily to prevent birth defects, especially spina bifida and other DCTNs (*CDC, 1992*). In 1999, the **Institute of Medicine** reaffirmed this recommendation as part of its baseline dietary intake assessment. (230)

A recent study, published by the **China-U.S.**, investigated the efficacy of folic acid in preventing DCTN in low- and high-risk populations. It was suggested that folic acid might be effective in preventing DCTN recurrence at daily doses of **4 mg**, as recommended by the **U.S. Public Health Service** (221) .

USE OF FOLIC ACID IN THE PERICONCEPTIONAL PERIOD AND ITS IMPACT ON THE PREVENTION OF DCTN

Italian authors conducted a study to determine how often pregnant women consumed folic acid during the periconceptional period (three months before and two months after conception). It was found that 0.1% of the participants had not taken folic acid, which resulted in cases of anencephaly in couples without risk factors. Likewise, it was identified that 4.1% of women consumed it before pregnancy, 12.3% during the first two months of gestation, and only 0.5% complied with the recommended intake during the entire periconceptional period. This study concluded that it is essential to increase efforts to promote folic acid consumption as a preventive measure against neural tube closure defects (NTCD) (220).

Despite the proven efficacy of folic acid in reducing DCTN, there is a proportion of cases that are not preventable with its administration. Recent research suggests that folic acid may have less protective effect in certain ethnic groups (231). In addition, it has been reported that its periconceptional use could also contribute to the prevention of other congenital defects, such as cleft lip and palate, limb defects, and urinary tract anomalies (232)

In a study conducted by Wehby et al., it was reported that adequate folic acid intake not only prevents DCTNs, but also reduces the risk of cleft lip and palate, reinforcing the importance of supplementation during the periconceptional period (233).

PROPOSAL

The main strategy for increasing folate levels among women includes dietary modifications, folic acid supplementation, and food fortification. Dietary modifications consist of increasing the consumption of foods rich in folates, which can raise folate levels

in the blood. However, it is recommended to encourage a balanced diet that contains sufficient natural sources of folates.

Supplementation with folic acid (FA) tablets or multivitamins containing folic acid appears to be the most accessible method of increasing folate levels compared to a change in dietary habits. Studies have shown that only 30% of women between the ages of 18 and 45 regularly consume vitamin supplements, (232). Many women start taking prenatal vitamins only after finding out they are pregnant, which is late for the prevention of neural tube closure defects (NTCD).

Food fortification with folic acid makes it possible to reach large populations without the need to modify individual behavior. This strategy has been shown to be more cost-effective than dietary changes or individual supplementation. In 1996, the Food and Drug Administration (FDA) selected products such as cornmeal, pasta, and rice to be fortified with FA. The approved level of fortification was 140 micrograms per 100 grams of cereal. With this strategy, the FDA estimated that women would consume an average of 100 micrograms of FA daily through fortified products (234).

While this level of fortification can prevent some DCTNs, it is not enough as the only strategy. To maximize their impact, combined strategies targeting all women of reproductive age should be implemented, thus ensuring effective prevention of DCTN.

Biochemically, methionine synthetase is a key enzyme in the elimination of homocysteine, a metabolite associated with the risk of DCTN and cardiovascular disease. Gulati et al. demonstrated that vitamin B12 stimulates the activity of this enzyme, suggesting that its supplementation could complement the effects of folic acid, (235). On the other hand, Brouwer et al. showed that a diet rich in citrus vegetables and fruits reduces plasma homocysteine levels. These foods, being good sources of folates, contribute not only to the prevention of cardiovascular diseases but also to the reduction of the risk of DCTN, (236).

DISCUSSION

This chapter has offered a comprehensive review of neural tube defects (NTD), nosological entities that represent a group of severe congenital malformations, resulting from a failure to close the neural tube during embryonic development (Williams et al., 2020). These abnormalities, which include anencephaly, encephalocele, and spina bifida, constitute a significant public health problem given their variable prevalence depending on geographic region, nutritional factors, and genetic predisposition. The findings presented here underscore the persistent relevance of DCTN as a significant public health problem,

driving the need for an increasingly deep understanding of its multifactorial etiology and the continued search for more effective strategies for its prevention and management.

A central point of this review has been the intricate cascade of molecular events that orchestrate neural tube formation. We have detailed how neural tube induction, morphogenesis, and closure are processes finely regulated by the convergence and interaction of various signaling pathways, including Wnt, Sonic Hedgehog (SHH), BMP, and FGF. It is crucial to note that disruption in any of these intricate mechanisms, whether due to genetic or environmental factors, can lead to a failure in the closure of the neural tube, manifesting itself in the spectrum of DCTNs. In particular, the phenotypic variability observed in DCTNs – from anencephaly to spina bifida and encephalocele – can be understood in part as reflecting the specific stage and region of the neural tube where neurulation is interrupted, and presumably, by the molecular pathways primarily affected in each case. For example, alterations in anterior neuropore closure, resulting in anencephaly, could be particularly linked to dysfunctions in specific signaling pathways that are dominant in the cephalic region during the final stages of cranial closure.

The etiology of DCTN is recognized as multifactorial, with a complex interaction of genetic and environmental factors. This review reaffirms the strong association between folic acid deficiency and an increased risk of DCTN, one of the most consistent findings in research on the prevention of these defects. Pioneering epidemiological studies have demonstrated the efficacy of preconceptional folic acid supplementation in reducing the incidence of DCTN, with an estimated decrease of up to 70% (Smithels et al., 1980; Medical Research Council, 1991). In addition to the leading role of folates, research has extended to other nutritional factors, such as vitamin B12 and homocysteine metabolism. Alterations in homocysteine metabolism have been implicated as contributors to the pathogenesis of DCTN (Brouwer et al., 1999), underscoring the interconnectedness of different metabolic pathways in early embryonic development. In addition to nutritional factors, environmental factors such as maternal exposure to teratogens – including certain anticonvulsants such as valproic acid and carbamazepine – poorly controlled maternal diabetes, and broader nutritional deficiencies should be considered (Wiznitzer et al., 2005).

Prenatal diagnosis of DCTN is based on a combined strategy that integrates high-resolution ultrasound and biochemical testing. Ultrasound has proven to be an effective tool for early detection, allowing anencephaly to be identified from the 11th week of gestation, and to visualize spina bifida and encephalocele more accurately around week 16 (Dawson et al., 2018). At the same time, alpha-fetoprotein (AFP) dosing in maternal serum and amniotic fluid persists as a fundamental pillar in prenatal screening, especially between

16 and 18 weeks of gestation. AFP values that exceed 2.5 times the median (MoM) for gestational age are considered abnormal and suggestive of an increased risk (López-Miranda et al., 1999). In cases of elevated AFP, amniocentesis and subsequent determination of acetylcholinesterase in amniotic fluid may provide a more accurate diagnosis, increasing the specificity of screening (Brock et al., 1972).

The prognosis of DCTN is variable and dependent on the type and severity of the defect. Anencephaly, unfortunately, presents itself as a lethal condition without effective therapeutic options. In contrast, spina bifida and encephalocele offer possibilities for surgical management, although clinical results are heterogeneous and depend on the degree of neurological involvement of the patient (Wilkins-Haug, 1999). Myelomeningocele, the most severe form of spina bifida, frequently coexists with Arnold-Chiari malformation type II and hydrocephalus (Taylor et al., 2018), imposing complex and long-term multidisciplinary management. Early surgical correction of myelomeningocele can improve quality of life, but most patients will require a comprehensive approach to address persistent motor, urinary, and orthopedic problems (Rodriguez et al., 2016). In the context of encephalocele, whose term etymologically comes from the Greek egkephalos (brain) and kelé (hernia) (Smith et al., 2005), treatment and prognosis vary significantly depending on the amount of herniated brain tissue and the location of the defect (Jones & Brown, 2012; Gómez & Martínez, 2017). Minor encephaloceles, particularly those covered with skin and located in the nasofrontal, nasoethmoidal and nasoorbital regions (Fernández & López, 2019; Jones & Brown, 2012), may have survival rates of 80-93% when the protrusion is anterior, but the prognosis becomes less favorable in occipital encephaloceles with severe central nervous system involvement (Fernández & López, 2019; Taylor et al., 2018). Clinically, encephalocele is frequently associated with mental retardation, seizures, and microencephaly (Rodriguez et al., 2016). In parietofrontal locations, sensory problems such as hearing and visual problems have been described, as well as nasal complications (Fernandez & Lopez, 2019). The severity of the symptomatology depends critically on the size and location of the defect, and the volume of brain tissue involved.

In the field of public health, folic acid supplementation and food fortification emerge as fundamental preventive strategies. The FDA's approval in 1996 of mandatory fortification of cereal-based products with folic acid represents a milestone in the prevention of DCTN, leading to a substantial reduction in incidence in the United States and other countries with similar policies (Food and Drug Administration, 1996). However, despite robust evidence of benefits, adherence to preconceptional folic acid supplementation persists suboptimal in numerous regions. Studies carried out in Italy, for example, reveal

that only 12.3% of pregnant women consumed folic acid during the first months of pregnancy, and less than 0.5% did so in the optimal periconceptional period (Rosch et al., 2005). Therefore, complementary approaches to optimize prevention, including inositol and vitamin B12 supplementation, are being explored, which have shown promising results in animal models and high-risk population studies (Brouwer et al., 1999).

While food fortification strategies and folic acid supplementation have achieved a significant reduction in the incidence of DCTN, substantial challenges remain in early detection, equitable access to antenatal care, and the identification of new preventive strategies. Promising emerging areas of research include the study of genetic polymorphisms associated with folate metabolism, which could explain individual variability in response to standard supplementation and guide more personalized strategies (Gulati et al., 2012). Research into epigenetics and its role in regulating neural tube closure opens up new avenues for innovative preventive therapies. Likewise, the development of gene editing strategies to correct genetic mutations associated with an elevated risk of DCTN represents a long-term research frontier. At the public health level, the implementation of preconception education and counseling programs emerges as a key strategy to improve adherence to folic acid supplementation, especially in high-risk populations.

CONCLUSION

This chapter has outlined the complex and multifaceted landscape of neural tube defects (NTDs), from the intricate molecular biology underlying neurulation to the spectrum of clinical and public health strategies designed to lessen its burden. Through an exhaustive review, the multifactorial nature of DCTN has been revalidated, where the confluence of a multifaceted genetic predisposition and specific environmental factors, with a preponderant role of folate deficiency, orchestrate the disruption of an ontogenetic process as critical as the closure of the neural tube.

It has been shown how research in molecular biology has elucidated the intricate signaling pathways – Wnt, Sonic Hedgehog (SHH), BMP, FGF – and the constellation of genes whose dysfunction can precipitate the appearance of DCTN, establishing a direct causal link between these fundamental mechanisms and the clinical heterogeneity that characterizes these pathologies. A detailed understanding of neurulation at the molecular level not only enriches the body of basic knowledge about human development, but also provides an essential conceptual framework for unraveling the complex etiology of DCTNs and for identifying therapeutic and preventive targets more accurately. Accordingly, for a comprehensive understanding of TNCD, it is essential to recognize and interpret the

anatomy and developmental biology of the nervous system, particularly the sequential and coordinated processes of primary and secondary neurulation (Smith et al., 2005). The accurate identification of the molecular and genetic factors involved in neural tube closure represents a significant advance that contributes to both earlier diagnosis and more effective prevention strategies for these abnormalities.

The scientific evidence compiled throughout this chapter unequivocally corroborates the protective role of folic acid. From pioneering studies that demonstrated the efficacy of preconceptional supplementation – reducing the incidence of DCTN by up to 70% (Smithels et al., 1980; Medical Research Council, 1991) – until the establishment of population-scale food fortification programs, folic acid emerged as a primary prevention tool with a notable impact on public health. However, it has been recognized that pharmacological prevention does not achieve absolute efficacy, and that challenges persist to achieve optimal adherence to periconceptional supplementation and to address the fraction of cases that do not respond to this intervention, which demands the exploration of complementary and personalized preventive strategies. In this context, it is crucial to know the multifactorial etiology and risk factors associated with DCTN, including, in addition to folate deficiency, vitamin B12, alterations in homocysteine metabolism (Brouwer et al., 1999), exposure to teratogens (Wiznitzer et al., 2005), and polygenic genetic factors, with the aim of providing more accurate and tailored dietary and genetic counseling to at-risk families (Jones & Brown, 2012).

In the clinical field, the prenatal diagnosis of DCTN has undergone substantial advances. The synergistic combination of high-resolution ultrasound, which allows detecting anencephaly from the 11th week of gestation and visualizing spina bifida and encephalocele accurately from week 16 (Dawson et al., 2018), and biochemical screening by alpha-fetoprotein (AFP) dosing in maternal serum and amniotic fluid with abnormal values defined as those exceeding 2.5 times the median (MoM) for gestational age (López-Miranda et al., 1999), are consolidated as essential diagnostic tools for early detection. When detecting an abnormality of the central nervous system using these techniques, it is recommended to exhaustively search for concomitant malformations in other systems or organs, thus optimizing the diagnostic strategy and avoiding the unnecessary practice of invasive procedures such as amniocentesis, and its associated cytogenetic study (Taylor et al., 2018). In cases of altered serum AFP screening, but without ultrasound confirmation of DCTN, it is essential to highlight the importance of alpha-fetoprotein (AFP) screening in maternal serum and amniotic fluid as a guiding tool in the detection of fetal anomalies, with definitive diagnostic confirmation being obtained by high-resolution ultrasound.

Amniocentesis and subsequent evaluation of acetylcholinesterase in amniotic fluid, on the other hand, are valuable complementary tests to confirm the diagnosis in equivocal or high-risk cases. It is essential, however, to provide reassurance to families and avoid generating unnecessary distress with preliminary serum screening results, remembering that a definitive diagnosis can only be established after a detailed ultrasound evaluation and, in some cases, additional confirmatory tests (237) (238) (239)

The clinical management of DCTN, particularly in the most severe forms such as myelomeningocele and complex encephaloceles, remains a long-term multidisciplinary challenge. Although anencephaly remains a lethal condition, spina bifida and encephalocele benefit from corrective surgical treatment and comprehensive multidisciplinary management, with heterogeneous results depending on the severity and location of the malformation, and the degree of neurological involvement. In this sense, it is crucial to highlight that the **prognosis** of DCTN intrinsically depends on the severity of the defect, with anencephaly being an unviable condition, while spina bifida and encephalocele can be addressed by surgery and multidisciplinary management to optimize quality of life, although with significant variations depending on the involvement of the nervous tissue and the location of the malformation.

Looking to the future, research at DCTN faces crucial challenges and opens up promising exploration horizons. To unravel more precisely the intricate genetic architecture of DCTNs, to understand the molecular basis of individual variability in folate response, to elucidate the role of epigenetic factors in the regulation of neurulation, and to explore the preventive and therapeutic potential of other nutrients and metabolic cofactors such as vitamin B12 and inositol, which are already showing promising effects, represent priority and strategic lines of research. Similarly, the emergence of gene-editing strategies to correct mutations associated with a high risk of DCTN foresees a future of targeted gene therapies. At the translational and public health level, it is (240) **necessary to strengthen public policies focused on preconception education**, to **actively promote supplementation with folic acid and other multivitamins with preventive effect**, and **to guarantee equitable access to efficient prenatal screening programs**, in order to maximize preventive impact at the population level. (239)

It is concluded that the knowledge of neural tube defects has undergone a remarkable advance in recent decades, allowing to improve the prevention, diagnosis and clinical management of these complex conditions. However, significant challenges remain in adherence to folic acid supplementation, the reduction of false positives and negatives in AFP screening, equitable access to quality health services, and the development of

innovative therapies that substantially improve the long-term prognosis. A robust multidisciplinary approach, integrating cutting-edge biomedical research, evidence-based public health education, and the formulation and implementation of effective and equitable public policies, stands as the cornerstone to continue reducing the incidence of these congenital disorders and tangibly optimizing the quality of life of affected individuals and their families.

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