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Research

Clinical and Molecular Characterization of Neural Tube Defects with Special Emphasis on MTHFR Gene

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Abstract

Background: Studies have highlighted that MTHFR plays a crucial and essential role in formation of neural tube in early fetal life, and thus occurrence of any mutation to this gene will eventually affected the normal process of neural tube formation leading to defects and the severity of it depends on type of mutation and its impact on clinical picture of patient having this congenital anomalies.

Objectives of the study: To describe demographic data and clinical presentation of Sudanese patients with neural tube defect. To characterize *MTHFR* gene in Sudanese patients with neural tube defects. To correlate clinical presentation with molecular findings of *MEHFR* gene.

Material and methods: This is a cross-sectional study that had been performed at National Center of Neurological Sciences (NCNS) during June 2016 to October 2019. The study included clinical data and peripheral blood sample taken from all neural tube defects patients diagnosed at NCNS, during the above mention period. The study was conducted in accordance with the guidelines of the local ethical committee. Blood samples were obtained from 50 neural tube defect patients. Blood samples were taken in sterile containers that contained EDTA and processed for DNA extraction. The extracted DNA was amplified by PCR for detection of MTHFR gene.

Results:

A total of 50 patients were studied, the linguistic affiliation of the studied patients showed that neural tube defects were common in Afro Asiatic tribes' mothers 42% and were 38% Nilo-Saharan. The common mother age group was 21-25 years 52%, followed by 31-35 years 34%. The clinical presentation regarding deficit was not favorable because 80 % of patients were having variable degrees of neurological deficits. The sequence results showed that, Insertion A was detected in one sample (lumber myelomeningocele) at 3 positions cDNA.113_114insA (ch1:11863189_11863190insT), cDNA.114_115insA (1:11863190_11863191insT) and cDNA.115_116insA (1:11863191_11863192insT) consecutively. Insertion A at those positions was at 5'UTR regions and changes were set as splice site changes. A>G was found in the same previous sample at position cDNA.92A>G (chr1:11863211T>C) and was predicted as polymorphism change at 5'UTR region a splice site change. A>T was detected in the same sample in position cDNA 1559A>T (ch1:11854064) resulting in polymorphism and amino acids sequence change at the CDS region. C>T was elicited in sample of patient with sacral meningiocele at position cDNA 246 (ch1:11863057) at CDS region resulting in polymorphism splice site change. rs (2066470) no amino acid changes.

Conclusion: In conclusion this study showed that female predominates and constitutes 60% of affected patient. The most common type of open neural tube defects was meningiomyelocele forming 88% of all affected patients and the most common site was Lumbo-sacral region. Strong correlation was detected between anatomical site and motor deficit, 34 patients with lumbo-sacarl myelomeningocele were having neurological deficit in form of bilateral lower limb weakness. The sequence results in this study showed that, Insertion A was detected in one sample in 3 positions and A>G was found and predicted as polymorphism change at 5'UTR region a splice site change, also A>T was detected in same sample resulting in polymorphism and amino acids sequence change at the CDS region. C>T was elicited in another sample at position cDNA 246, at CDS region resulting in polymorphism splice site change, with no amino acid changes.

***Keywords:** MTHFR Gene, Molecular characterization, Neural tube defects*

1.1 Introduction

Neural tube defects (NTDs) are known to be a group of congenital anomalies characterized by defects in posterior midline structures, including skin, bone, dura and neural tissue. Spinal dysraphism refer to those anomalies affecting bony and/or nervous components of the spine. This anomaly is believed to be due a failure of or incomplete, neural tube closure during different embryologic stages.

This is a process that normally occurs during the third to fourth week of fetal life. It can involve any part of the spine, but commonly it involves the Lumbo-sacral spine. Management of spinal dysraphic anomalies involves a number of steps: accurate diagnosis; an assessment of the severity of the lesion; a decision whether intervention is warranted; the nature of the surgical intervention; and educating the Family of the need for lifelong medical care.

1.2 Overview of neural tube defects

Neural tube defects form a major category of congenital abnormalities of fetal spine. Neural tube defects results from failure of closure of the spinal neural tube. The pathological process is not that clear process; however, there is a clear bond between folic acid deficiency and a variety of structural abnormalities of which karyotype or genetic defects have been reported.(1,2,4)

A large number of terms have been used to describe neural tube defects and led to the confusion regarding its nomenclature. Spinal dysraphism is one of these terms and includes all forms of spina bifida regardless cranial or spinal location, this term is used by both patients and health care providers inconsistently to describe myelomeningocele specifically or other spinal dysraphic anomalies generally. 'spina bifida' term is synonymous term to spinal dysraphism. One classification scheme for dysraphic spinal condition divides these anomalies into two major general categories: one is closed defects which are known also as spina bifida occulta, and other open spinal defects also known also as spina bifida aperta.

Prognosis is strongly dependent on the type of the defect and the presence or absence of additional structural or genetic mutations. Open defects carries poor prognosis regarding the presence of neurological motor or sensory deficits and resultant disability and mortality. Closed defects have a good prognosis comparison to open neural tube defects (1, 2).

1.3 Incidence of neural tube defects.

Neural tube defects incidence In the United States is approximately 1-2 cases per 1000 live births, whereas the incidence in the UK and Europe is about four times greater than this (22). The most common form of open neural tube defects is myelomeningocele, in which there is a segment of anatomically and structurally abnormal spinal cord and nervous tissues that are exposed in the midline of the back through a bony defect in the vertebral column and overlying skin (1).

In lesser form deformity, meningocele which is a sac-like structure composed of only meninges without involvement of the underlying neural tissues.(4). Closed forms of spinal neural tube defects are those anomalies in which the underlying midline defects, hidden by intact skin. Simple spina bifida occulta is the congenital absence of posterior elements of the spine. Cutaneous lesions include skin tags, hemangiomas, lipomas, hairy patches and skin dimples. (5) The other malformations included in this category are lipomyelomeningocele, fatty filum terminale, dermoid and epidermoid cysts, and dermal sinus tract and split cord malformations. These lesions are usually diagnosed in early infancy, but if the cutaneous manifestations are subtle, diagnosis may be missed until symptoms develop many years later. Regardless of that Surgical repair of these lesions is often delayed but it's the final destination of treatment. (5)

1.4 Embryology of spinal cord

The embryology of the spine is a complex process because the spine function is not only structural support of the body, but it also serves as a safe passage of the neural elements, alterations in any of the following e embryologic steps can result in one or more congenital abnormalities of the spine (6). Neurulation is a term used to define the formation of the neural tube and is the process Which finally leads to the development of the brain and spinal cord and it has two stages, primary and secondary neurulation. In the embryo it starts when the flat neural plate is formed, which is composed of ectoderm, folds along the dorsal midline into the neural groove. This is followed by fusion of the two edges of the groove in midline, resulting in a closure of the tube which is then turn covered by a layer of cutaneous ectoderm. (7, 9)

The first stage the primary neurulation forms the spinal cord only to the segments corresponding to the lower lumbar level. Abnormalities at specific points during primary neurulation lead to various forms of dysraphism such as myelomeningocele, lipomyelomeningocele, intraspinal dermoid and epidermoid cysts, and split Cord malformations. This process takes place during the period from day 18 to day 28 of embryonic development. (.8, 9)

The second stage of this process is called Secondary neurulation. This process occurs during days 28 to 48 of embryonic development and is divided into two steps, canalization and regression (9). During canalization, a mass of undifferentiated cells caudal to the neural tube and notochord assembles into a structure called the caudal cell mass. A canal within the caudal cell mass connects with the rostral neural tube formed during primary innervation and ultimately forms the distal segments of the spinal cord (below L2).

Errors during canalization can lead to a terminal myelocystocele or lipomyelomeningocele. (8) Regression refers to the process by which most of the caudal spinal cord arising from the caudal cell mass with the exception of the conus medullaris and nerve roots involutes, leaving a thin and nonfunctional filum terminale. Failed or inaccurate regression can lead to a fatty filum terminale. (8, 9)

After formation of the terminal filum, the vertebral canal grows at a faster rate than the neural tube, This differential growth results in the relative 'ascent' of the spinal cord, such that at the time of birth, the conus medullaris lies at the L2-L3 level, and reaches the normal adult level of theL1-L2 interspace by 3 months of age. (8)

In trials to analyze the origin of both open and closed NTDs during neural tube formation of the mouse, mutant loop-tail (*Vangl2* gene) has shown that craniorachischisis, the most severe anterior neuropore NTDs, results from failure of Closure 1. Most commonly, embryos complete Closure 1 but fail in later enervation, presenting NTDs as separate open lesions of the cranial neural tube (exencephaly, progressing to anencephaly) and/or spinal neural tube (open spina bifida) (15). The wave of 'zippering' closure down the body axis can arrest at any stage, results in an open spina bifida of varying length. Hence, *Zic2* mutant mice fail early in spinal enervation, owing to lack of dorsolateral neural plate bending; these mice exhibit a large spina bifida from thoracic level downwards. In contrast, spinal closure in the curly tail (*Grhl3*) mutant fails later, due to enhanced axial curvature of the body axis. This produces a spina bifida confined to the lumbo-sacral region. When secondary innervations is disturbed, closed defects occur commonly at sacro-coccygeal levels ('spinal dysraphism') in which the spinal cord is characteristically 'tethered' to adjacent tissues, reflecting faulty tissue separation during tail bud development (15,16).

1.5 Clinical prospective of neural tube defects

Clinical severity of NTDs varies according to extent of neural elements involvement for example Encephalocele can be lethal depending on the extent of brain damage during herniation; Open spina bifida defects is generally compatible with life and postnatal survival, although they result in neurological deficit below the level of the lesion can lead to lack of sensation, inability to walk and incontinence of the sphincters (10).

Associated conditions such as hydrocephalus, which often requires cerebrospinal fluid diverting procedures, vertebral deformities, and genitourinary and gastrointestinal disorders, all previously mentioned associated anomalies had an impact on degree of clinical severity and disability (10). Closed spinal lesions are generally less severe and can be asymptomatic, such in case of spina bifida occulta which is considered a variant of normal (1, 2).

1.6 Factors predispose to occurrence of neural tube defects

Genetic and non-genetic factors are involved in the causative factors of NTDs, with majority of NTD prevalence due to genetic factors. Evidence of genetic involvement includes an increased risk occurrence in siblings (2-5%), compared with the 0.1% risk in the general population, (11).Women with affected pregnancies has a higher risk of recurrence (10%). NTD prevalence is greater in similar-sex twins if they are thought to be monozygotic one, compared with unlike-sex twins, consistent with a significant genetic component (11, 12). Despite those facts, occurrence of multiple cases in families is very rare. (12).

Up to date, genes in two main areas of molecular biology have yielded positive findings with regard to NTD etiology: folate one-carbon metabolism and non-canonical Wnt signaling (the planar cell polarity pathway) (13).

Variety of non-genetic factors have been studied and found to have association with human neural tube defects. This involves wide variety of teratogenic agents known to cause the defects in rodents, of particular clinical significance is valproic acid (VPA), which increases the risk of NTDs 10-fold when taken during early in pregnancy stages (17). The teratogenic mechanisms underlying anticonvulsant action may involve anti-folate effects, particularly for carbamazepine, recent findings with VPA suggest a potent histone deacetylase (HDAC) inhibitory activity (18). This may lead to disturbance of balance of protein acetylation versus deacetylation, similar to the action of the HDAC inhibitor, trichostatin-A, which causes NTDs in mice (18). Further environmental teratogen with proven effect in humans is the fungal product fumonisin, which was responsible for a 2- fold increase in NTD prevalence along the Texas-Mexico border in the early 1990s (19).

Fumonisin is a potent NTD-causing teratogen in mice, with marked effects on spingolipid metabolism that likely disturbs downstream embryonic gene expression (19). Other 'environmental' factors implicated in the etiology of NTDs include maternal diabetes, maternal obesity, and exposure to high temperatures during early pregnancy (20).

It is essential to keep in mind that environmental causes of birth defects are most likely the most preventable predisposing factors of neural tube defects, environmental causative factors form only a small proportion of all congenital disorders that have a known cause, estimated at 0.12 cases per 1000 births (0.5% of all defects) in a recent survey of European pregnancies are caused by environmental cause (5).

NTDs comprise a diversity of birth defects that are considered to occur during the third and fourth week's post-fertilization, (2). Nevertheless, we have a limited understanding of the concept of cellular and molecular mechanisms by which human NTDs arise during early embryonic development period (9). On the other hand, as genetic risk factors start to emerge from modern genomics research, it is vital to be able to understand when and how such gene variants might exert their effects (3). Similarly, it is important to appreciate the precise embryonic mechanisms that might be the targets of therapeutic interventions (21).

1.7 Pre-natal diagnosis of neural tube defects

Two approaches have been used to screen NTDs in low-risk populations which are biochemical testing of maternal blood for alpha-fetoprotein (AFP) and the use of traditional 2D or 3D ultrasound. Some screening programs combine the two techniques. (23) NTDs Detection rates by routine ultrasound examination should reach 100% for open neural tube defects due to the presence of the easily recognizable cranial and spinal signs (24).

1.8 Genetics of neural tube defects

More than 200 genes studied are known to cause NTDs in mice, but there is limited progress in studying the molecular basis underlying most human NTDs. Many genetic studies have been run to investigate candidate genes in cohorts of patients, with particular reference to those that participate in folate one-carbon metabolism. Although the homocysteine remethylation gene MTHFR has emerged as a risk factor in some human populations (3).

Because of the tight bond and historical relationship between folic acid and NTDs, it is not weird that folate pathway genes have been most intensively studied. Positive associations have been reported between specific folate-related gene variants and NTDs in a number of case-control studies. For example, methylenetetrahydrofolate reductase (*MTHFR*) encodes a key cytoplasmic enzyme of folate metabolism, which generates 5-methyl tetrahydrofolate for homocysteine remethylation (3, 13). The *MTHFR* polymorphism C677T (rs1801133) is associated with a 1.8-fold increased risk of NTDs. Another significant risk factor is the R653Q variant (rs2236225) of *MTHFD1*, a trifunctional enzyme that catalyses the conversion of tetrahydrofolate to 5, 10 methylenetetrahydrofolate (3).

Recently, genes that encode enzymes functioning in mitochondrial one-carbon metabolism have also been considered influencing NTD etiology. An intronic polymorphism in *MTHFD1L*, the gene for mitochondrial 10-formyl-THF synthetase, is associated with increased risk of NTD (14). Therefore, genetic variants that reduce the efficiency of folate one-carbon metabolism increase the risk of NTDs. This may indicate that the mitochondrial contribution to folate metabolism is particularly relevant and/or sensitive in terms of mammalian neural tube closure (13, 14). At time of innervations, the embryo undergoes lengthening and narrowing of the initially disc-shaped neural plate in order to make sure that the neural folds are well spaced for closure to begin. This lengthening of the neural plate and underlying mesoderm mandates a lateral to medial displacement and intercalation of cells, called convergent extension. Molecular level wise, convergent extension cell movements are dependent on non-canonical Wnt signaling: the planar cell polarity (PCP) pathway (15).

Possible involvement of the PCP pathway in human NTDs came from the discovery of PCP gene involvement underlying severe NTDs in several mouse mutants. Mutations in the trans-membrane proteins *Vangl2*, *Celsr1*, *Ptk7* and *Fzd3/6* (double mutant), and the cytoplasmic proteins *Dvl1/2/3* and *Scrib*, resulted in craniorachischisis, a severe form of NTD in which failure of closure of neural tube occur along most of the body axis, ending with long segment defect which may start an from midbrain and end in sacral spine (15, 16). the finding of non-synonymous amino acid changes in affected humans and not in control samples, although suggestive, but does not prove their causal role in the NTDs. More evidence is needed to demonstrate whether the specific human 'mutations' actually cause protein dysfunction or reproduce the NTD phenotype in an animal model (15).

Ciruna et al wrote in his report that up to date, assays of PCP protein function including interaction with Dishevelled and translocation to the plasma membrane have identified functional defects in NTD-associated variants of *VANGL1*, *VANGL2*, *CELSR1* and *SCRIB*. Several *VANGL1* missense variants block the rescuing effect of *Vangl1* mRNA on the *Vangl2* (trilobite) mutant phenotype in zebrafish. (16).

1.9 MTHFR gene

MTHFR mutations are the major studied genetic risk factor that might result in NTDs. Complementary DNA for human *MTHFR* was first isolated in 1994, by reverse- transcriptional PCR using degenerate oligonucleotides that were synthesized on the basis of peptide sequence information from the purified porcine liver enzyme. (26). 5, 10- methylenetetrahydrofolate reductase (*MTHFR*) enzyme is involved in metabolism of folate. The *MTHFR* gene is located on chromosome 1 (1p36.3), and two common alleles, the C677T (thermolabile) allele and the A1298C allele, also were described (26, 27)

The population frequency of C677T homozygosity ranges from 1 % or less among Blacks from Africa and the United States to 20% or more among Italians and US Hispanics. (25) Function methylenetetrhydrofolate reductase catalyzes the conversion of 5,10- methylenetetrhydrofolate to 5-methyltetrahydrofolate, cofactor for homocysteine remethylation to methonine (26).

Human MTHFR gene composed of 11 exons and a human cDNA for MTHFR, 2.2 kb in length, has been expressed and shown to result in a catalytically active enzyme of approximately 70 kDa. Fifteen mutations have been identified in the MTHFR gene: 14 rare mutations associated with severe enzymatic deficiency and 1 common variant associated with a milder deficiency. The common polymorphism has been implicated in three multifactorial diseases: occlusive vascular disease, neural tube defects, and colon cancer (26.27).

Goyette et al identified 2 missense mutations and 1 nonsense mutation in 1994 and also described additional 7mutations in late 1995 (28).

1. 10 Problem statement

Neural tube defects are common pathology among Sudanese newborns with congenital abnormalities, despite that fact, ante-natal mother education and early diagnosis is still a dilemma in our healthcare system.

1.11 Justification

Care for patients with neural tube defects depends on strict ante-natal follow up, early detection of the defect in utero and Clinical evaluation in post-natal period with early surgical intervention alongside with basic assessment of gene mutations, all this steps will add cumulative knowledge and will help family and community to deal in good way with their affected member and be part of the treating multidisciplinary team as needed. Findings of this research will provide base to establish the best way managing those patients depending on new parameters in our healthcare system.

1. 12 Objectives

1. 12.1 General Objective

To study the Clinical and Molecular Characterization of Neural Tube Defects with Special Emphasis on *MTHFR* Gene among Sudanese patients.

1. 12. 2 Specific Objectives

1. To describe demographic data and clinical presentation of Sudanese patients with neural tube defect
2. To characterize *MTHFR* gene in Sudanese patients with neural tube defects.
3. To correlate clinical presentation with molecular findings of *MEHFR* gene.

2.1 Material and Methods

This is a cross-sectional study that was performed at the National Center for Neurological Sciences (NCNS) – Khartoum - Sudan in a time frame from June 2016 to September 2017. All the operated and non operated pediatric patients (50 patients), clinically, and radiologically (Radiological investigations included brain CT scan, US and MRI spine) diagnosed as having neural tube defects, during the above stated period were included in the present study. Clinical and demographic data was collected prospectively from each participant utilizing a structured questionnaire; in addition the laboratory data was obtained from PCR findings. The variables included (age, sex, tribe, consanguinity, residence, clinical presentation, anatomical site of neural tube defects, presence or absence of motor deficit ,associated hydrocephalus, associated congenital anomalies, family history of similar condition ,ante-natal care, folic acid supplement, intrauterine diagnosis and outcome of delivery radiological tools, treatment, outcome and PCR product of *MTHFR* gene). The ethical approval was obtained from the ethical committee at the National Center for Neurological Sciences.

2.2 DNA extraction and purification

Genomic DNA was isolated from peripheral blood leukocytes by the standard phenol chloroform extraction method; 10 ml of RCLB was added to 2.5 ml of blood then centrifuged for 5 minutes at 6000 rpm, this step was repeated until a clear pallet of white blood cell appeared, the supernatant was discarded and 2ml of WCLB, 1 ml of Guanidine Hydrochloride, 300 µl of ammonium acetate and 10 µl of proteinase K were added and incubated at 37ºC overnight. Next day the mixture was cooled to room temperature and 2 ml of pre-chilled chloroform was added, the mixture was vortexes then centrifuged for 5 minutes at 6000 rpm after that, the upper layer containing DNA was collected to a new test tube and 10 ml of pre-chilled Absolute Ethanol was added and kept at - 20ºC for 2 hours. After incubation the precipitated DNA was centrifuged for 10 minutes at 6000 rpm, after that the supernatant was discarded then the pellet was washed in 4 ml of 70% ethanol then the pellet was centrifuged for 10 minutes at 6000 rpm, after centrifugation the supernatant was poured off and the pallet was left to dry overnight. The DNA was dissolved in 100 µl of ddH2O then vortexes, incubated at 4ºC for 24 hours and stored at -20ºC till utilization.

2.3 Methyltetrahydrofolate reductase (MTHFR) gene amplification

The gene was amplified by PCR. The following primers sequences were used to obtain a fragment 233 bps (rs).

Forwards: 5′ CTGCCACTCAGGTGTCTTGA 3′

Reverse: 5′ TCTTCTCCCGGAGTCTCTCA 3′

In a PCR test tube, 14 µl ddH2O, 4 µl of master mix containing (1.5 buffer, nM MgCl2, 200µm of dNTPs and 0.5 units of Taq polymerase) were added, and then 1 µl from each primer and 2 µl of genomic DNA were added. The PCR was carried out using a commercial thermal cycler (Swift™ MaxPro SWT-MXP-BLC-4). The amplification steps included an initial 4 minutes of denaturation at 94 °C, followed by 35 cycles of denaturation at 94 °C for 30 seconds, the primers were annealed at 60.5°C for 30 secs, then the elongation period was 30 seconds at 72 °C , after that, the final elongation was adjusted for 5 minutes at 72 °C.

2.4 Visualization of PCR product

The PCR amplification product was separated on a 3 % agarose gel and trans-illuminated with UV light with a 100-base-pair ladder.

2.5 Sequencing

PCR products were sent for sequencing to BGI-solutions Hong Kong Co. Ltd.

2.6 Data analysis

The data was analyzed using Statistical Package for the Social Science (SPSS) version 25 software program; the statistical methods included the frequencies & percentages for the quantitative variables and the Mean & SD for the qualitative variables. In addition the study detects the relation using the correlation test & the Chi square test, the p value <0.05 was accepted to be statistically significant.

2.7 Bioinformatics

The chromatogram sequences were visualized through BioEdit software version7.2.5. The nucleotides sequences of the studied genes were searched for sequences similarity using nucleotide BLAST NCBI (https://www.ncbi.nlm.nih.gov/) and subjected to multiple sequence alignment using BioEdit software version7.2.

3.1 Demographic Data Results

In this study females were 30 constituting 60% and male were 20 constituting 40%. (Table 1) The most common age presentation was infant group (1 month - <1 year) of 29 constituting (58%), followed by neonates (<1month) 18 (36%) and rest of results were showed in (table 2). Forty of the patients showed positive consanguinity between their parents (80%); 31 were of first degree cousin marriage (77.5%) and the rest were of second degree cousin marriage 9 (22.5 %). (Table 3). Regarding the residence of origin 21 (42%) patients are from western states followed by central states 15 (30%), northern states 9 (18%), the rest of results were showed in (Table 4). Afro-asiatic tribes were the most common among mother tribes constituting 21 (42%), followed by Nilo-Saharan tribes 19 (38%), Nigro-Congo tribes constituting 10 (20%). (Table5).

3.2 Clinical Results

The most common anatomical site of NTDs was Lumbo-sacral region 37 (74%), followed by occipital 4 (8 %), and least commonly the cervical region 1 (2%), rest results were showed in (Table 6). All patients presented with open type of neural tube defects 50 (100%), (Table 7). With regard to open neural tube defects the commonest type was meningiomyelocele 44(88%), followed by encephalocele 4 (8%) and the least common type was meningiocele 2 (4%) (Table 8).

Motor deficit was the most common clinical presentation with 41 (80%) of patient having deficit and 9 (20%) patient with no deficit (Table 9). Both lower limbs motor deficit was the most elicited motor deficit 39 (78%) and the rest results were showed in (Table 10). the commonest power grading in limb weakness was grade II 14 (34.1%), followed by grade 0 13(31.7%), grade I 9(22%) and finally grade III 5 (12.2%) (Table11).

In this study patients of neural tube defects presented with sphincters disturbances were 2 (4%) and they presented with urinary retention, while the rest 48(96%) there was no urine retention nor constipation and it was difficult to assess incontinence .families were not trained or practicing intermittent catheterization 50 (100%).mile stones were delayed in 5 (10%) of patients and normal in 45 (90%). (Table12). Most of the patients were under school age 49 (98%) and only 1 (2%) was at age of school but didn't enter it.

Regarding psychosocial behavior it was difficult to be assessed in 36 (72%), normal in 12(24%) and abnormal in 2 (4%). Neural defects were associated with hydrocephalus in 29(58%) patients and not in 21 (42%). (Table13) Onset of hydrocephalus was commonly since birth 28(93.3%), and at time of presentation in onset of hydrocephalus was commonly since birth 28(93.3%), and at time of presentation in 2 (6.7%) (Table14).

Congenital anomalies other than Chiari malformation type 2 which was present in all patients of meningomyelocele 88%, were not associated with NTDs 47(94%), and only associated in 3(6%) (Table 15), limb deformities were the only other associated deformities in this study 3(6%). Only 2 (4%) patients were involved in multidisciplinary team care the rest 48(96%) were not involved. (Table16). Family history of similar condition was only positive in 1 (2%) patient in a second degree cousin. Single patient was attached to rehabilitation center 1 (2%) the rest were not attached 49 (98%) (Table17).

All patients with neural tube defect underwent MRI scan for the site of defect 50(100%).regarding brain scan , 6 did both MRI and CT brain 12%, 42 did CT-brain 84%, 2 did MRI-brain 4%.

The common mother age group was 21-25 years 26(52%), followed by 31-35 years 17 (34%), the rest of results were showed in (Table 18). 21 (42%) mothers were Para II, followed by Para III 15(30%), rest of results were showed in (Table 19). History of miscarriage was not noticed in 39(78%), and there in 11(22%).

Mothers on regular ante-natal care follow up visits were 36(72%), and who are not are 14 (28%). (Table 20) all mothers were not exposed to known teratogenic drug, radiation, insecticides nor were passive or active smokers 50(100%).

Folic acid supplementation was appropriate in 23 (46%) mothers, followed by partial 15(30%) then non in 12 (24%) (Table 21).

Intrauterine diagnosis was established in 5 (10%) and not in 45(90%) (Table 22). Ultrasound was the only modality of intrauterine diagnosis (100%). Mode of delivery was mainly normal spontaneous vaginal delivery 35(70%) and caesarean section in15 (30%). Single outcome of delivery was in 47(94%) and affected twins were 3 (6%) (Table 23). Two identical twins were affected and single twin of the non-identical twins was affected.

3.3 Cross-tabulation Results

There was no correlation between age, motor deficit and the power grading of the deficit P- value (0.223 and 0.160) respectively. There was a strong correlation between Age and milestone of patients, 26 infants and 17 neonates were having normal mile stones P-value 0.047. There was no correlation between age and onset of hydrocephalus because all affected patients were having hydrocephalus since birth P-value (0.800) Strong correlation was detected between anatomical site and motor deficit P value =0.000,34 patients with lumbo-sacral myelomeningocele were having deficit. There was no correlation between anatomical site and Sphincters disturbances P-Value =0.981 There was no correlation between anatomical site and onset of HCP P-value 0.161 with regards to associated anomalies there was no correlation between anatomical site and other congenital anomalies P-value 0.462 (Table 24). There was strong correlation between Age and attachment to rehabilitation center p-value 0.000 (Table 25).

Table 1: Shows frequency of sex among Sudanese patients with NTDs

Frequency of Sex	Frequency	Valid Percent
Female	30	60.0
Male	20	40.0
Total	50	100.0

Table 2: Shows frequency of age among Sudanese patients with NTDs

Frequency of Age	Frequency	Valid Percent
Neonate (<1month)	18	36.0
Infant (1 month - <1yr)	29	58.0
Toddler (1-3 yrs)	1	2.0
Preschooler (>3-5yrs)	1	2.0
School age (6-12 yrs)	1	2.0
Total	50	100.0

Table 3: Shows frequency of consanguinity among parents of Sudanese patients with NTDs.

Frequency of Consanguinity	Frequency	Valid Percent
Yes	40	80.0
No	10	20.0
Total	50	100.0

Table 4: Shows frequency of residence among Sudanese patients with NTDs.

Frequency of Residence	Frequency	Valid Percent
Central states	15	30.0
Eastern states	3	6.0
Northern states	9	18.0
Western states	21	42.0
Southern states	2	4.0
Total	50	100.0

Table 5: Shows frequency of mother tribe of Sudanese patients with NTDs.

Frequency of Residence	Frequency	Valid Percent
Afro-Asiatic	21	42.0
Nilo-Saharan	19	38.0
Niger-Congo	10	20.0
Total	50	100.0

Table 6: Shows frequency of anatomical site of NTDs among Sudanese patients.

Frequency of Anatomic Site	Frequency	Valid Percent
Occipital	4	8.0
Cervical	1	2.0
Dorso-Lumbar	3	6.0
Lumbar	3	6.0
Lumbo-Sacral	37	74.0
Sacral	2	4.0
Total	50	100.0

Table 7: Shows frequency of type of NTD among Sudanese patients.

Frequency of type of NTD	Frequency	Valid Percent
Open	50	100.0
Total	50	100.0

Table 8: Shows frequency of type of open NTD among Sudanese patients.

Frequency of Residence	Frequency	Valid Percent
Meningiocele	2	4.0
Encephalocele	4	8.0
Meningiomyelo-cele	44	88.0
Total	50	100.0

Table 9: Shows frequency of motor deficit among Sudanese patients with NTDs.

Frequency of Motor deficit	Frequency	Valid Percent
Yes	41	80.0
No	9	20.0
Total	50	100.0

Table 10: Shows frequency of Localization of motor deficit among Sudanese patients with NTDs.

Frequency of Localization of Motor deficit	Frequency	Valid Percent
One limb	1	0.25
Both lower limbs	39	99.50
Four limbs	1	0.25
Total	41	100.0

Table 11: Shows frequency of power grading in limbs weakness among Sudanese patients with NTDs

Frequency of Power grading in Limbs weakness	Frequency	Valid Percent
Grade 0	13	26.0
Grade I	9	18.0
Grade II	14	28.0
Grade III	5	10.0
Total	41	100.0

Table 12: Shows frequency of mile stones delay among Sudanese patients with NTDs.

Frequency of milestone delay	Frequency	Valid Percent
Normal	45	90.0
Delayed	5	10.0
Total	50	100.0

Table 13: Shows frequency of associated hydrocephalus among Sudanese patients with NTDs.

Frequency of Associated Hydrocephalus	Frequency	Valid Percent
Yes	29	58.0
No	21	42.0
Total	50	100.0

Table 14: Shows frequency of Onset of associated Hydrocephalus among Sudanese patients with NTDs.

Frequency of Onset of Associated Hydrocephalus	Frequency	Valid Percent
Since birth	28	93.3
At time of presentation	2	6.7
Total	30	100.0

Table 15: Shows frequency of Associated other congenital anomalies among Sudanese patients with NTDs.

Frequency of Associated other congenital anomalies	Frequency	Valid Percent
Yes	3	6.0
No	47	94.0
Total	50	100.0

Table 16: Shows frequency of Multidisciplinary team care involvement among Sudanese patients with NTDs.

Frequency of Multidisciplinary team care involvement	Frequency	Valid Percent
Yes	2	6.0
No	48	94.0
Total	50	100.0

Table 17: Shows frequency of Attachment to rehabilitation center among Sudanese patients with NTDs.

Frequency of Attachment to Rehabilitation center	Frequency	Valid Percent
Yes	1	2.0
No	49	98.0
Total	50	100.0

Table 18: Shows frequency of mother age among Sudanese patients with NTDs.

Frequency of Mother age	Frequency	Valid Percent
=< 20 yrs	5	10.0
21-25 yrs	26	52.0
26-30 yrs	2	4.0
31-35 yrs	17	34.0
Total	50	100.0

Table 19: Shows frequency of mother parity among Sudanese patients with NTDs.

Frequency of Mother parity	Frequency	Valid Percent
Para I	6	12.0
Para II	21	42.0
Para III	15	30.0
> Para III	8	16.0
Total	50	100.0

Table 20: Shows frequency of regular ante-natal care among mothers of Sudanese patients with NTDs.

Frequency of Regular Ante-natal care	Frequency	Valid Percent
Yes	36	72.0
No	14	28.0
Total	50	100.0

Table 21: Shows frequency of folic acid supplementation among mothers of Sudanese patients with NTDs.

Frequency of Folic acid Supplementation	Frequency	Valid Percent
Appropriate	23	46.0
Partial	15	30.0
None	12	24.0
Total	50	100.0

Table 22: Shows frequency of intrauterine diagnosis of NTD among Sudanese patients.

Frequency of Intrauterine Diagnosis of NTD	Frequency	Valid Percent
Yes	5	10.0
No	45	90.0
Total	50	100.0

Table 23: Shows frequency of outcome of delivery among Sudanese patients with NTDs.

Frequency of Intrauterine diagnosis of NTD	Frequency	Valid Percent
Single	47	94.0
Twins	3	6.0
Total	50	100.0

Table 24: Shows cross-tabulation between anatomical site of neural tube defects and motor deficit among Sudanese patients with NTDs.

Anatomical Site of NTD	Motor deficit	Motor deficit	Total
	Yes	No	
Occipital	1	3	4
Cervical	0	1	1
Dorso-Lumbar	3	0	3
Lumbar	2	1	3
Lumbo-Sacral	34	3	37
Sacral	0	2	2
Total	40	10	50

Table 25: Shows cross-tabulation between age and attachment to rehabilitation center among Sudanese patients with NTDs.

Age	Attachment to Rehabilitation center	Attachment to Rehabilitation center	Total
	Yes	No	
Neonate (<1month)	0	18	18
Infant (1 month - <1yr)	0	29	29
Toddler (1-3 yrs)	1	0	1
Preschooler (>3-5yrs)	0	1	1
School age (6-12 yrs)	0	1	1
Total	1	49	50

3.4 PCR Results

Positive PCR was detected in all blood samples, furthermore 5 PCR products (10 reactions) from patients and apparently healthy individuals were sent for sequencing. Figures (1)

3.5 Sequencing Results

The sequence results showed that, Insertion A was detected in female neonate presented with lumber myelomeningocele, mother age was between 31-35 years and she is Para 3 and was not on ANC and not on folic acid supplement. (sample A) at 3 positions cDNA.113_114insA (ch1:11863189_11863190insT), cDNA.114_115insA (1:11863190_11863191insT) and cDNA.115_116insA (1:11863191_11863192insT) consecutively. Insertion A at those positions was at 5'UTR regions and changes were set as splice site changes. (Figure 3)

A>G was found in the same sample A at position cDNA.92A>G (chr1:11863211T>C) and was predicted as polymorphism change at 5'UTR region a splice site change. (Figure 4)

A>T was detected in sample A in position cDNA 1559A>T (ch1:11854064) resulting in polymorphism and amino acids sequence change at the CDS region. (Figure 5) C>T was elicited in female neonate coming , he presented with Sacral Meningiocele and no motor deficit. Mother Para 1 was on regular ANC follow up visits and appropriate folic acid supplement (sample B) at position cDNA 246 (ch1:11863057) at CDS region resulting in polymorphism splice site change. rs (2066470) no amino acid changes. (Figure 6) chromograph detected C>T in MTHFR gene in sample B at position cDNA 246 (ch1:11863057) at CDS region resulting in polymorphism splice site change (Figure 7).

Enrichr showed a significant association between MTHFR gene and folate metabolism pathways.

(Figure 8) in addition an association between the gene and spina bifida x-linked, abortions, spina bifida folate sensitive, spina bifida aperta of cervical spine and neural tube defects x-linked diseases. (Figure 9)

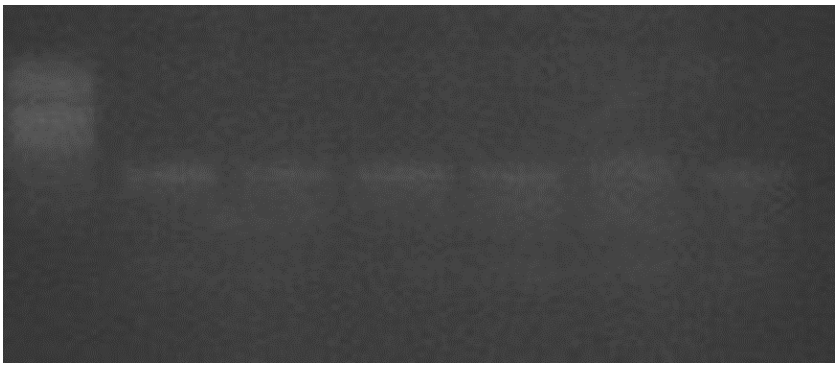

Figure 1. *Shows gel electrophoresis of MTHFR gene in neural tube defects patients, lane 1(100bp ladder), lane (2, 3, 4, 5, 6 and 7) 233 bp of MTHFR gene.*

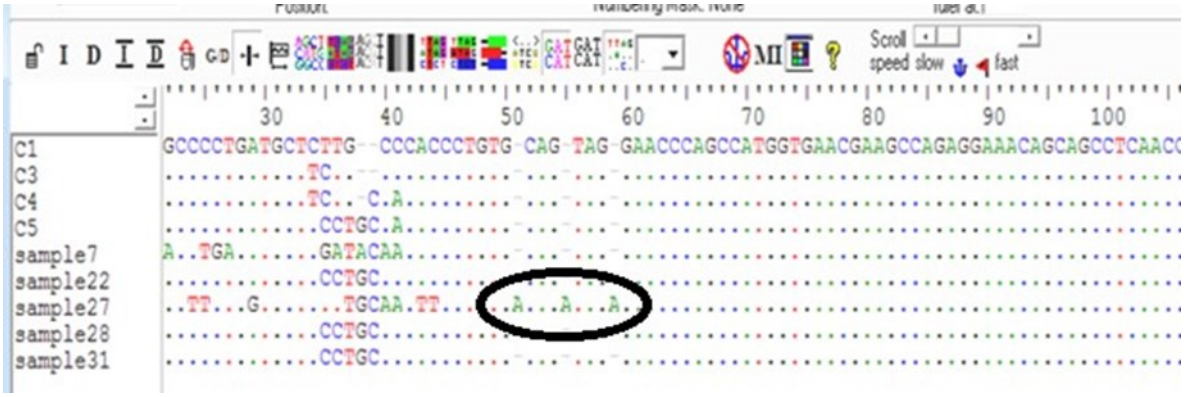

Figure 2. *Shows the alignment of PCR sequence results with the reference sequence of MTHFR gene, Insertion A was detected in sample (A) at 3 positions.*

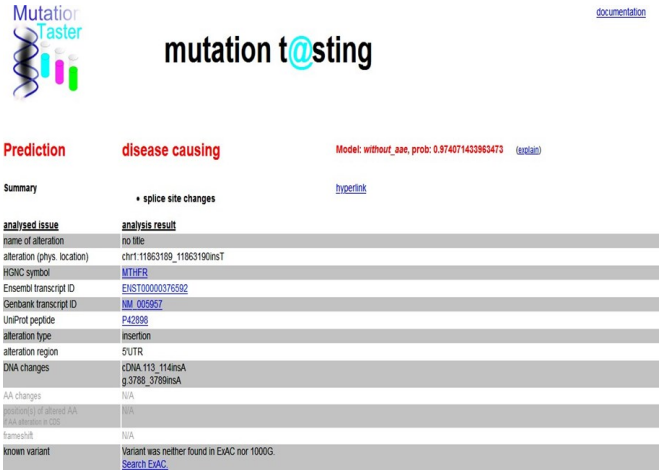

Figure 3. *Shows mutation testing for insA in MTHFR gene in sample A*

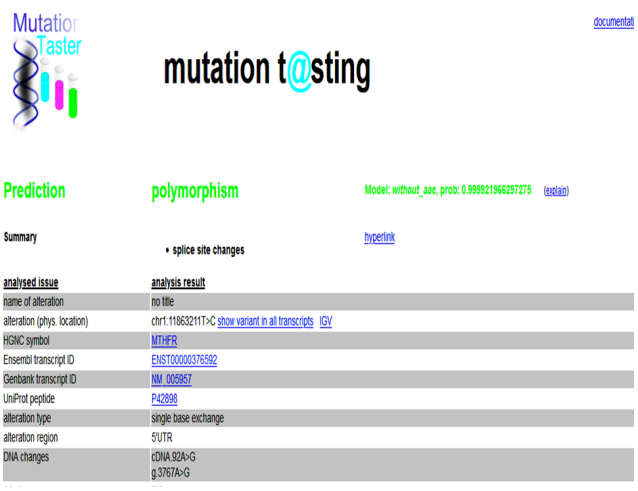

Figure 4. *Shows mutation testing for A>G in MTHFR gene in sample A at position cDNA.92A>G (chr1:11863211T>C) and was predicted as polymorphism change at 5'UTR region a splice site change*

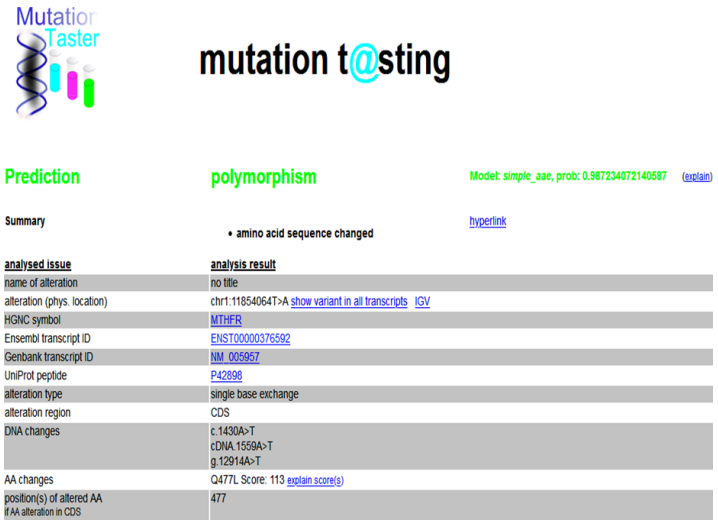

Figure 5. *Shows mutation testing of A>T in MTHFG detected in sample A in position cDNA 1559A>T (ch1:11854064) resulting in polymorphism and amino acids sequence change at the CDS region.*

Figure 6. *Shows a mutation detecting C>T in MTHFR gene in sample B at position cDNA 246 (ch1:11863057) at CDS region resulting in polymorphism splice site change.*

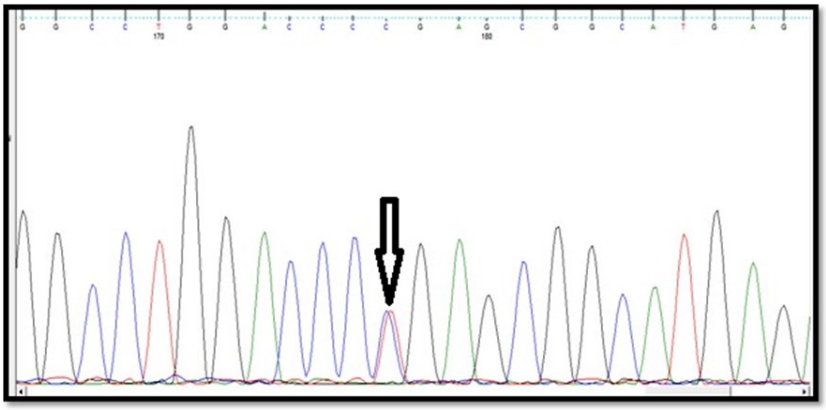

Figure 7. *Shows a chromograph detecting C>T in MTHFR gene in sample B at position cDNA 246 (ch1:11863057) at CDS region resulting in polymorphism splice site change.*

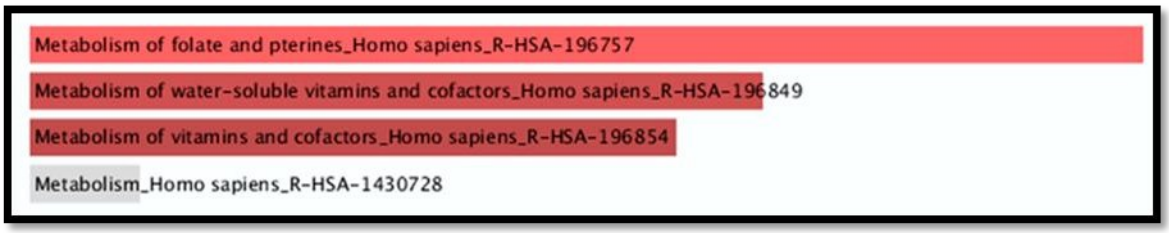

Figure 8. *Shows Enrichr pathways link with MTHFR gene (p-value 0.008).*

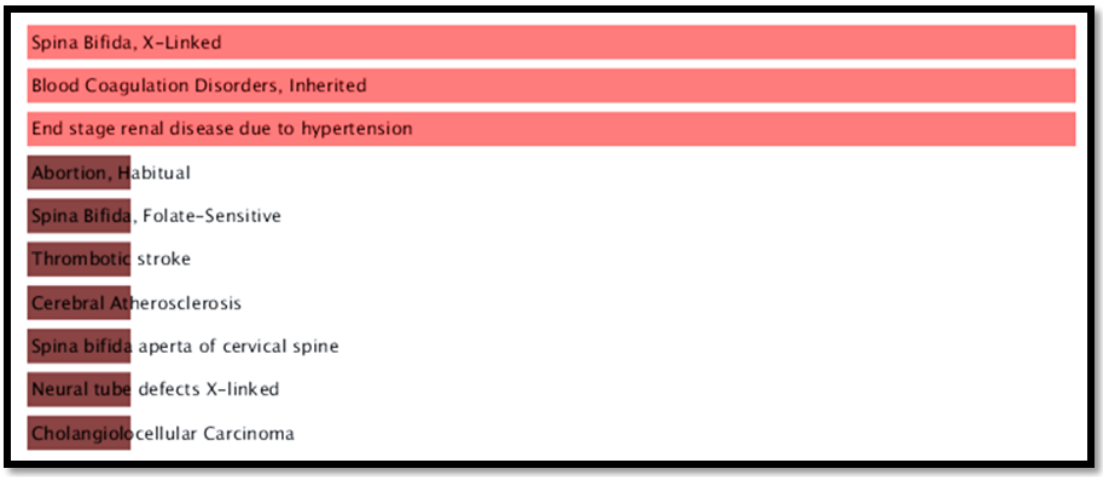

Figure 9. *Shows Enrichr disease link with MTHFR gene.*

4.1 Discussion

This study was done to evaluate the clinical status and characterization of MTHFR gene among Sudanese patients with neural tube defects.

Our findings showed that female were predominant 30 patients (60%)in study done in western Iraq, the number of babies born with NTDs was 33 (11 Males and 22 females), giving an overall incidence of 3.3/1000 live births with a male to female ratio of 1:2 (31). Buccimazza et al published paper in 1994 studied the prevalence of neural tube defects in cape town which showed that, there was a female preponderance for both spina bifida (M: F ratio 0. 89) (32). There was also Turkish study which showed a total of 66 cases with a NTD were recorded in 21,907 births. Prevalence rate of NTDs was 30.1 per 10,000 births. Of these 66 cases, 29 (43.9%) were male and 37 (56.1%) female. Female/male ratio was 1.27 (33), so this study result is Contrary to many worldwide studies that showed female predominance. The distribution of presentation age of studied patients showed that 58% of patients were aging between 1 month and < 1 year and 36 % were below 1 month of age, this finding is not of a clinical significance rather than being indicator of late exposure of neural tube defect patients to health care providers in Sudan.

In this study there was statistically no significant correlation between mother tribe and tendency to have neural tube defects. The Afro-Asiatic tribes constituted 42 %, Nilo- Saharan were 38 % and Niger-Congo 20 %. This finding point stressed on that there is no tribe relevance to occurrence of neural tube defects as many studies stated that fact. In fact in this study there are states which contain large number of patients in comparison to other states, western Sudanese states comprise 21 (42%) patients, followed by central states 15 (30%) patients.

Regarding the anatomical site of neural tube defects, the most common affected region was Lumbo-sacral region in 37 patients (74%) followed by occipital 4 (8%), and this findings are consistent with local study findings in NCNS Khartoum Sudan which showed that Lumbo-sacral area were the commonest affected site (34.6%) (34).

The most common type of neural tube defects elicited was meningiomyelocele 88% this finding is consistent with local study findings done in 2014 in NCNS Khartoum Sudan which stated that Myelomeningiocele is the commonest form of neural tube defects in Sudan (34). Another two studies findings from Saudi Arabia and Ghana were consistent with our study result the first one from Saudi Arabia involved 42 patients with neural tube defects showed that Eighty-three percent of the cases had myelomeningocele (MMC), 12% had encephalocele, and 2.5% had meningocele (35). The second study titled Prevalence of neural tube defect and hydrocephalus in northern Ghana Among the spinal bifida cases, myelomeningocele occurred in 13 patients (59.1%), with meningocele occurring in 8 patients (40.9%) (36) Neurological deficit was very frequent finding 80% of patient having deficit specifically bother lower limbs 99.50%, in comparison to other studies these findings are common especially in Lumbo-sacral myelomeningiocele and dorsal myelomeningiocele.

Mile stones were normal In 90% of patients affected with neural tube defects, motor deficit was prominent and affecting progression of normal mile stone, cognition was difficult to assess due age in most of the patients.

Twenty nine patients (58%) of neural tube defects were having associated hydrocephalus 90% of them are myelomeningiocele patients; study in Burkina Faso showed thirty-eight cases were included; there were 27 cases of Spina bifida and 11 cases of encephalocele associated with hydrocephalus. A cerebral CT scan was performed in all patients. In 30 cases, the operative management of these pathologies was performed at the same operative time. (37), another study from Saudi Arabia showed that Hydrocephalus affects the majority of patients with spinal open effects who have Myelomeningocele (MMC) and CM II and requires close surveillance and prompt management (39).

Twenty eight (93.3%) patients of neural tube defect were having associated hydrocephalus since birth other studies showed that almost more than 80% of patients with Chiari type 2 develop hydrocephalus since birth (38).

Associated other congenital anomalies were very few with only 3 (6%) patients having limb deformity. Regarding similar studies one multi-centric study showed that isolated NTDs constitute the majority of the studied patients which is different from the results obtained in a study from Riyadh (40), where syndromic, genetic (mainly inherited as autosomal recessive), and chromosomal defects were more prevalent than in other populations, and constituted around 20% of total NTDs (41).

The age of mothers of patient of neural tube defects was commonly 21-25 years old in 52% of mothers followed by 31-35 years 34%, according to worldwide studies for example one done in cape town, South Africa showed that the highest NTD rates were found at both ends of the maternal age range (<20 years and >35 years of age) (32).

Frequency of regular ante-natal care was predominant with 36 (72%) mothers involved in this kind of care , this percent in comparison to local study done in Sudan in 2018 found that 28% of mothers included in the study reported that they had never received ante-natal care (42).Other study from Pakistan in Lady Reading hospital Peshawar between July 2013 and January 2014 showed that 23.73% of mothers have no idea of antenatal care, 34.32% mothers were not having any history of antenatal care, and only 22.38% were having a prenatal visit in the first trimester of pregnancy, 25 mothers were not having history of folate intake and only 8 (11.94%) were having positive history of taking in the first trimester. 18/67 (26.86%) of the affected children were the first child and the rest were the second or third (43).

Folic acid supplementation was appropriately given for 23 (46%) mothers ,partially in 15 (30%) and 12 (26%) were not taking this supplement at all ,in study done in Khartoum through 2014-2015 studied mothers regarding folic acid supplement 68 (66%) of the studied mothers received folic acid during pregnancy with the current child, of those who received folic acid 66 (97.1%) started medication after conception, 36 (54.5%) started in the first trimester and 39 (57.4%) had irregular intake of folic acid (41).

Intrauterine diagnosis of patients with neural tube defects was infrequent with only 5 (10%) cases have been diagnosed with ultrasound only.

One of the interesting findings is that two identical twins were having open type of neural tube defect, other non-identical twin was also involved but his other twin was a healthy one, in study made by Mastroiacovo., et al he stated that twin pregnancies are at an increased risk of congenital anomalies compared with singleton pregnancies (29).

This is a fact of twin pregnancies which is complicated by neural tube defects (NTD) in our case. Sebire et al in his published paper in 1997 stated that the prevalence of NTD in twins is 2.3/1,000 in UK, These anomalies are seen in both monozygotic (MZ) and dizygotic (DZ) twin pairs, reflecting the multifactorial pattern of inheritance (30).

The *MTHFR* gene is located on chromosome 1 (1p36.3), and two common alleles, the C677T (thermolabile) allele and the A1298C allele, also were described (26, 27) Function methylenetetrhydrofolate reductase catalyzes the conversion of 5, 10- methylenetetrhydrofolate to 5-methyltetrahydrofolate, a cofactor homocysteine remethylation to methonine (26).

Human MTHFR gene composed of 11 exo ns and a human cDNA for MTHFR, 2.2 kb in length, has been expressed and shown to result in a catalytically active enzyme of approximately 70 kDa. (26.27).

Our sequence results showed that, Insertion A was detected in female neonate presented with lumber myelomeningocele, whom her mother was not on regular folic acid supplement her age range was between 31-35 years old at 3 positions. Insertion A at those positions was at 5'UTR regions and changes were set as splice site changes.

A>G was found in the same previous sample at position cDNA.92A>G and was predicted as polymorphism change at 5'UTR region a splice site change. Also, A>T was detected in the same sample in position cDNA 1559A>T, resulting in polymorphism and amino acids sequence change at the CDS region.

C>T was elicited in female neonate coming Central states, Afro-Asiatic linguistic affiliation, she presented with Sacral Meningiocele and no motor deficit and her mother was on regular ANC and folic acid supplement at position cDNA 246, at CDS region resulting in polymorphism splice site change. No amino acid changes.

Enrichr showed a significant association between MTHFR gene and folate metabolism pathways; in addition, an association between the gene and spina bifida x-linked, abortions, spina bifida folate sensitive, spina bifida aperta of cervical spine and neural tube defects x- linked diseases.

Goyette et al Identified 3 substitutions in the MTHFR gene: 2 missense mutations and 1 nonsense mutation, the nonsense mutation and 1 of the missense mutations (Threonine to Methionine) were identified in severe early-onset patients; the second missense mutation (Arginine to Glycine) (26).

Frosst et al in paper published in 1995 identified a C-to-T substitution at nucleotide 677 that convert an alanine to a valine residue and are responsible for the synthesis of a thermolabile form of MTHFR (44).

In 1996 Motulsky revised the possible role of homocysteine level elevation in MTHFR polymorphism in neural tube defects (45). Mills and colleagues study which published in 1995 showed that mothers of infants with neural tube defects showed high level of homocysteine (46).

Ou et al data suggest that the 677C→T polymorphism of the MTHFR gene is a risk factor for both spina bifida and anencephaly that may provide a partial biologic explanation for why folic acid prevents these types of NTD (47).

Christensen and his colleagues in their study in 1999 stated that A polymorphism in the gene encoding methylenetetrahydrofolate reductase (MTHFR), 677C→12;T, is the first genetic risk factor for NTDs in man identified at the molecular level (48).

Our results showed a substitution mutation A>G in the sample A at position cDNA.92A>G (chr1:11863211T>C) and was predicted as polymorphism change at 5´UTR region a splice site change. No registered mutation was found in the database in this position, and this mutation is significant as its affect on the 5´UTR, which is also known as a leader sequence or leader RNA. It is the region of mRNA that is directly upstream from the initiation codon and it is essential for the regulation of translation of a transcript into a protein product which then regulate the translation of the main coding sequence of the mRNA.

However, the 5′ UTR is completely untranslated, it has been found to interact with proteins relating to metabolism; and proteins translate sequences within the 5′ UTR. In addition, this region has been involved in transcription regulation linked to mRNA export.(49) And as our resultant mutation 5′UTR effect analysis showed its significant regulatory features on H3K9me1, Histone and Histone 3 Lysine 9 mono-methylation; the histone family 3 a group of histone proteins that regulate the condensation of the DNA genetic material forming the chromatin in the term of nucleosomes units, further more regulating the process of DNA transcription and translation due to the regulated binding and un-binding effect of these histones. And as this binding of the DNA to these histones is regulated by certain reactions one of which is methylation that is affected due to the impact of this mutation, the consequent functions of these proteins will be disrupted. (49,50)

4.2 Conclusion

In conclusion this study showed that female predominates and constitutes 60% of affected patient. The most common type of open neutral tube defects was meningiomyelocele forming 88% of all affected patients and the most common site was Lumbo-sacral region. Strong correlation was detected between anatomical site and motor deficit, 34 patients with lumbo-sacarl myelomeningocele were having neurological deficit in form of bilateral lower limb weakness. The sequence results in this study showed that, Insertion A was detected in one sample in 3 positions and A>G was found and predicted as polymorphism change at 5'UTR region a splice site change, also A>T was detected in same sample resulting in polymorphism and amino acids sequence change at the CDS region.

C>T was elicited in another sample at position cDNA 246, at CDS region resulting in polymorphism splice site change, with no amino acid changes.

4.3 Recommendation

We recommend in this study:

1. To Increase awareness among mothers and community about possible causes of neural tube defects.
2. To improve pre-natal care measures by increasing number of facilitated centers and well trained medical personnel.
3. To develop the concept of treating the patient as multidisciplinary team throughout his life and include mother education in this task.
4. To do more comprehensive studies on *MTHFR* gene to detects other possible mutations and direct all efforts toward improving patient quality of life.

Abbreviations

NTD: Neural tube defects

MTHFR: Methyltetrahydrofolate reductase

HCP: Hydrocephalus

EDTA: Ethylenediamine tetra-acetic acid

DNA: Deoxyribonucleic acid

RNA: Ribonucleic acid

CT scan: Computerized tomography scan

cDNA: Complementary DNA

Bp: Base pair

mRNA: Messenger Ribonucleic Acid

MRI: Magnetic resonance imaging

kDa: Kilo Daltons

PCR: Polymerase chain reaction

RCLB: Red Cell Lysis Buffer

WCLB: White Cell Lysis Buffer

Rpm: Round per minute

ddH: Double-distilled water

VPA: Valproic acid

HDAC: Histone deacetylase

AFP: Alpha-feto protein

THF: Tetrahydrofolate

MMC: Meningiomyelocele

CDS: Coding sequence

UTR: Untranslated region

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