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Identification of novel mutation in VANGL1 gene in patients with myelomeningocele

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Abstract

Background: Neural tube defects are one of the most common birth defects. The most frequent forms of neural tube defects are anencephaly and myelomeningocele (Spina bifida) which occurs due to incomplete closure of neural tube at the cranial and caudal end respectively. Infants with anencephaly die in utero or are still born. Among all VANGL1 has been shown to be in close relation with the disease**.**

The present study aimed to investigate the mutation in VANGL1 gene among Pakistani population having myelomeningocele.

Materials & Methods: The study was conducted among fifty diagnosed cases of myelomeningocele with age group from 0-10 years. Several anatomical patterns were analyzed like site and size of the cyst, by prenatally on ultrasound. While taking history several parameters were considered like folic acid consumption by the mothers and the consequences of the disease on siblings. Blood samples of the patients were drawn and PCR was performed for VANGL1 gene mutations were identified by DNA sequencing.

Results: In this study we came across the variation of the disease which was more prevalent in males. Most common site was the lumbar region, showing the size of the cyst. On further investigation, it revealed that about 96% of mothers did not take folic acid during their pregnancy and 1.4% of patients showed positive family history having myelomeningocele. During our study we came across the rare mutation of VANGL1 in five patients. The mutations identified in VANGL1 gene which were at different substitution position of amino acid i.e. valine 239 with Phenylalanine.

Conclusion: With this study we have finally concluded that in future further investigations and diagnosis for neural tube disorders should be carried out. In other cases although history revealed myelomeningocele, mutation should be identified. So keeping this point in consideration, other gene mutation should be done and more research of same area based should be carried out.

Keywords: DNA sequence, gene mutation, myelomenigocele, Spina bifida, VANGL1

1. Introduction

Neural tube defects (NTDs) constitute one of the most common congenital abnormalities in humans, occurring in 1 per 1,000 live births [1]. It is an anomaly of central nervous system (CNS) which occurs due to inappropriate closure of neural tube [2]. The two common forms of NTDs are anencephaly and spina bifida. A condition in which some parts of either skull or brain remain undeveloped is called anencephaly [3]. Spina bifida is a defect in vertebral arches due to improper closure of neural tube and usually the neural tube closes within 28 days after fertilization. Neural plate is an extended slip per like patch of thickened epithelial cells of dorsal ectoderm having wide cranial and narrow spinal region on the surface ectoderm thickening is due to inductive signal from primitive node and notochord [4, 5]. The subsequent changes in the appearance of cell and alignment of epithelium will lead to lateral folding of neural plate [6]. This will result in the formation of neural groove in the center with projection on both sides which is called as neural fold. The neural tube will later on form the brain and spinal cord. The neural tube has two regions i.e. the cranial and the caudal region. The cranial end forms the brain and if remain open will lead to anencephaly. The caudal end forms the spinal cord and if unable to close will lead to spina bifida [7]. The prevalence of occulta is 10-20% and most of the time the cases are diagnosed accidentally on radiographs. Meningocele is a bony defect of spine which occurs due to protrusion of meninges and cerebrospinal fluid. It is not associated with any disability since the nervous system remains intact [5]. Myelomeningocele (MMC) is a condition in which the meninges along with the neural elements protrude through a sac. It is a life threatening state due to involvement of nervous system. An individual

may present with several other disorders like hydrocephalus, paralysis of legs either partial or complete and atonic bladder [8]. Studies have shown that maternal folate intake is associated with NTD [9]. The incidence of NTD has been reduced since 1990 by raising maternal folate consumption. The United States Food and Drug Administration have made folic acid mandatory in cereals and grain products. It has been seen that folic acid although had lessen the incidence of NTD but was unable to remove it completely [10]. Maternal hyperthermia if occurs in early pregnancy has shown to be a risk factor for NTD [11]. Women consuming high intake of caffeine during early pregnancy have increased risk of baby with spina bifida [12]. The occurrence of spina bifida in Pakistan is 3-5 per 1000 live births out of which meningocele is 5% and that of myelomeningocele is 95% [13]. It has been reported that myelomeningocele is more common in females [14]. Both the environmental and genetic factors are involved in the development of Myelomeningocele [15]. Molecular studies show that nearly 250 genes were associated with NTD [16]. The mutation which was identified were *CELSR1, Disheveled 2&3, FZD6, SCRIB,* and *Vangl1* [17, 18, 19, 20].

Materials and Methods

The patients were selected from the out patients department (OPD) of Jinnah Post Graduate Medical Centre (JPMC), Karachi, Pakistan. After physical examination, MRI and family history was taken. The blood samples of 50 diagnosed cases of MMC were taken. 2c.c. blood was drawn by a trained phlebotomist after taken informed consent. Samples were collected from J.P.M.C. in a period of 6months. DNA was extracted from whole blood by using spin column method. DNA extraction was performed from whole blood by QiAmp DNA Mini Kit. The extraction method was followed according to the protocol provided by the manufacturer. It is followed by PCR amplification of VANGL1gene. The PCR was carried out in a tube containing 20 µl of a reaction mixture made up of the following components: 10 pmol of each forward and reverse primers for VANGL1 gene, 500 µM of four deoxynucleotides, 2 U of Taq polymerase (Promega), 10 x PCR buffer containing and 1.5 mM $MgCl_2$. The thermal cycler (Master Gradient PCR System, Eppendorf AG, Germany) was programmed to first incubate the sample for 10 minute for 95°C followed by 35 cycles consisting of 94°C for 30s, 64°C for 1 minute and 72°C for 1 min with final extension for 10 minute at 72°C. The PCR amplified products were identified by electrophoresis on a 2% agarose gel, stained with ethidium bromide, and evaluated under transilluminator. The sizes of PCR amplified product were estimated according to the migration pattern of a 50bp DNA ladder (Gibco BRL Life Technologies). The amplified products were visualized and photographed using DOC gel documentation system (Vilber System). The amplified products were of 404base pairs. The samples were then sent for sequencing. Mutation analysis was done by aligning the sequence with the reference sequence

Results

The diagnosed cases of myelomeningocele were collected and examination was done. The lump was found on the vertebral column most commonly on the lumbar region among the diagnosed cases of MMC. As myelomeningocele is a congenital anomaly, therefore 94% of the patients were infants i.e. within 1 year of age. In these cases, cysts were mostly found on the lumbosacral region. However, in some cases cysts were found at cervical and thoracic regions as shown in figure 1a and 1b.

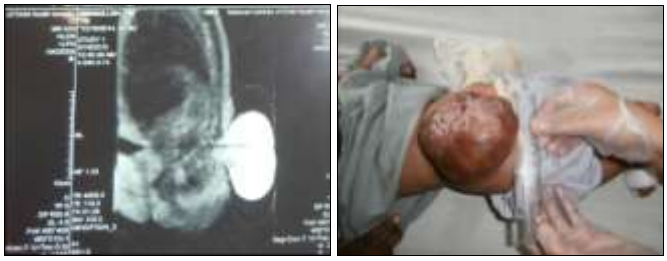

Fig (1a): A two month old boy having MMC cyst at lumbosacral region. **(1b)** MRI of the same boy showing MMC cyst.

The study was further carried out for the molecular analysis to look for the any novel VANGL1 mutations at the targeted region. In this instance sixty blood samples were collected after taking informed consent. Fifty children were diagnosed cases of MMC whereas ten were healthy which were taken as controls. DNA was extracted from the whole blood and PCR was performed and then the products were sent for commercial sequencing. Out of fifty samples one of them showed mutation in the targeted region of exon 4 of VANGL1gene and amplification was observed at 404 base pairs (bp) as shown in figure 2.

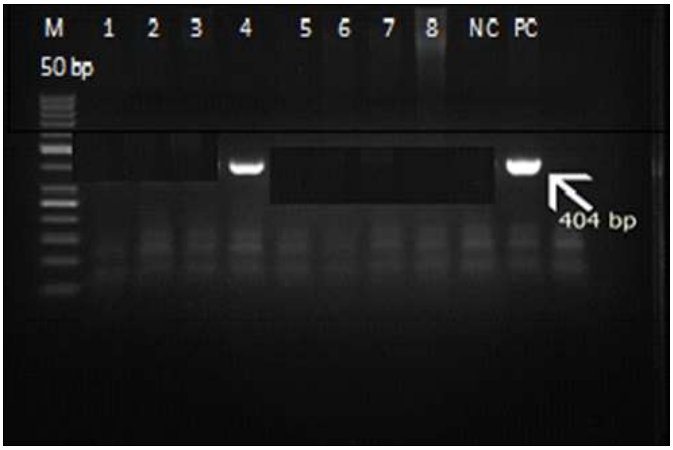

Fig 2: The analysis of VANGL 1 exon 4 DNA sample, Lane 1. 2 3, 5,6,7, and 8 were negative with the VANGL 1 exon 4 mutation whereas Lane 4was identified as positive for VANGL exon 4 mutation Lane N is negative control, Lane P is positive control and Lane M is the DNA ladder of 50 bp.

The mutation V239F was present in the targeted region of exon 4 of VANGL1 gene and this mutation was confirmed by DNA sequencing. Our predicted result showed the substitution of valine with phenylalanine at position 239 in sample and in our study for VANGL1 was novel mutation as it has not been reported earlier as shown in figure 3.

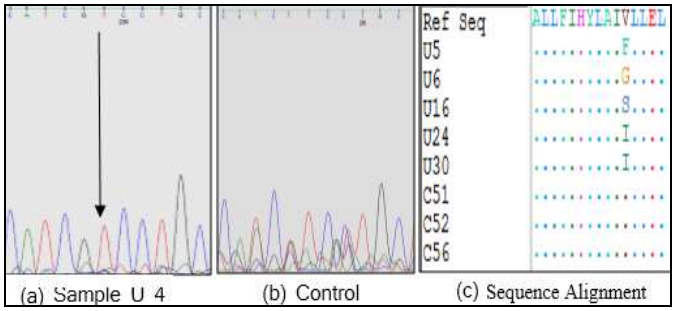

Fig 3(a): Shows the substitution of valine with phenylalanine at position 239 in sample number 4 compared with control **(b).** Sequence Alignment showing substitution of Valine to Phenylalanine confirmed by DNA sequencing **(c).**

The first mutation (V239F) was identified in sample # 4. He was a two month old boy had myelomeningocele cyst at lumbosacral region. No sign of neural tube defect is seen in any of his family member. His mother did not take folic acid during pregnancy. She was told by the concerned doctor about the disability of the baby prenatally.

Discussion

Congenital anomalies are the major cause of infant mortality. Among all the anomalies, 10% of infant mortalities are due to nervous system abnormalities [20]. MMC is the most common neural tube defects which occur due to improper closure of neural tube. It has been reported that the incidence of NTD varies in 1-2 per 1000 live births [21]. Patients present with various complications like lower limb weakness and loss of bladder control. It has been found that MMC is linked with several other congenital anomalies like hydrocephalus.

In our study of fifty patients of MMC we found that most of the patients have cyst on the lumbar region. Chand MB *et al* in 2011 also supported the feature of lumbar region being the most common site [22]. In 2013 Tamburrini *et al* also favored the concept of having the lumbar region as the most common site for MMC [23]. While reviewing the studies, we found that genetics also play a key role in the causation of the disease. In our study, while taking the history and filling the proforma, it was found that most of the mothers did not take folic acid during their pregnancy. Salomao, *et al* in 2017 suggested folic acid deficiency as prime factor of causing MMC [24]. This statement was agreed with the work of Yang *et al* in 2016 [25] and which was also favored by Gong *et al* in 2013 [26], as well as earlier in 2013 Copp AJ *et al* also supported that folic acid deficiency being the leading cause of MMC [27]. Apart from environmental factors, several genes are also involved in the etiology of MMC. Among all, VANGL1 has been shown to be in close association. In this cohort (n=50) patients, we identified a mutation in VANGL1 gene (p.val 239 Phe). This mutation modified evolutionary conserved amino acid residues in the membrane area of the VANGL1 protein and predicted functional consequences. We consider most likely that these are pathogenic mutation that may display a mutant phenotype under certain conditions (in a multifactorial setting). Since V239I prevents interaction of VANGL1 with DVL1, hence it stops molecular signaling at the time of gastrulation and neural tube closure. Bartsch, *et al* in 2012; identified some rare mutations for VANGLI gene and he found different mutation and substitution of amino acid which was reported. VANGL1mutation analysis in 144 unrelated individuals with NTDs from Slovakia, Romania and Germany and identified 3 heterozygous missense mutations: c.613G 1 A (p.Gly205Arg) with an open spinal bifida (lumbosacral meningomyelocele), c.557G 1 A (p.Arg186His) with a closed spinal bifida (tethered cord and spinal lipoma) and c.518G 1 A (p.Arg173His) with an unknown NTD. The c.613G 1 A mutation was also found in a healthy sibling. None of the mutations were described previously [28].

Conclusion

In this study we concluded that the rare mutation of VANGL1 gene is present in our population. During our study we also concluded the pathological consequences of the disease state. In this study not only the molecular data was presented but also the baseline anatomical and radiological data was also highlighted in connection to myelomeningocele.

An important finding of our study was the rare mutation of VANGL1 gene is present in our population. With this study we have finally concluded that in future further investigations and diagnosis for neural tube disorders should be carried out. In other cases although history revealed myelomeningocele but mutation was not identified. So keeping this point in consideration, other gene mutation should be done and more research of same area based should be carried out.

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